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AUSTRALASIAN ANNALS OF MEDICINE

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# THE CONCEPT OF LEUCHÆMIA[1]

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In giving you my views as to the concept of leuchæmia, I do not intend to embark upon a survey of the world literature or to give a standard lecture upon the many, some of them complex, hæmatological aspects of the disease, its classification and relation to other blood disorders, or to express the views, the hopes, the disappointments or the enthusiasms of the many who have written on the subject.

But having been interested in leuchæmia for twenty-five years, and having, because of that interest, been called upon to give advice in more than my share of cases of this deadly affliction, I shall present my impressions of the disease as judged from my own experience, and these will include observations on manifestations, diagnosis, prognosis, course and treatment. The order in which these matters are presented may be rambling and even illogical, nor do I anticipate that I shall be able to tell you anything new. But there are many occurrences in association with leuchæmia which are recorded as isolated facts in text-books of medicine without any emphasis upon the conclusions which can be drawn from them, and without any attempt to bring them into a perspective where they can be used to discuss the nature of the disease.

The spectacular and absorbingly interesting hæmatological changes have received the most minute attention, and this fact alone has tended to reduce careful detailed study of morbid anatomy and basic pathology, as well as to divert attention from certain clinical facts which may provide the clue to the elucidation of cause and the devising of appropriate treatment. The development of modern methods for studying cell growth and cell metabolism has also thrown further light on these problems by revealing the nature and potentialities of the leuchæmic cells, which are usually regarded as undoubtedly malignant on account of the invariable fatality of the disease, and, in the clinical field, on account of the obvious superficial resemblance to cancerous conditions.

[1] Received on July 31, 1956.

## CLINICAL CONTRASTS BETWEEN LEUCHÆMIA AND MALIGNANT DISEASE

All leuchæmias are characterized by an abnormal proliferation of leucopoietic tissue in the bone marrow, the proliferation being at an immature level which usually, but not always, results in the appearance of large numbers of leucocytes in the peripheral blood. Many of these leucocytes are immature, and the degree of immaturity bears a rough relationship to clinical severity. The most acute and fulminating clinical types are characterized by a preponderating if not an exclusive presence within blood of cells so primitive that they often defy exact differentiation. The same applies to the terminal phases of the chronic types which, finally, are often acute from both the clinical and the hæmatological viewpoints. These well-known facts should give food for thought. In malignant disease in general, we recognize acute fulminating growths, such as the mesoblastic sarcomata, and at the other end of the scale the chronic long-standing schirrus types of carcinoma. This, then, is common ground with leuchæmia. But it is difficult to think of examples of orthodox malignant disease in which the chronic schirrus type suddenly and terminally takes on fulminating characters.

We know also that leuchæmia may remit spontaneously, or from the intrusion of other complications or as the result of treatment,

and I would remind you that in other malignant diseases spontaneous remission is so rare that when it occurs it raises grave doubts as to the correctness of the original diagnosis. Somehow or other an explanation must be found for the remissions in leuchæmia which, though they do not always occur, are nevertheless a well-recognized feature of leuchæmia and seem to separate the disease from the orthodox neoplastic processes.

Leuchæmia, in common with malignant disease of any kind, is characteristically accompanied by anæmia. This feature, indeed, is often the presenting symptom, as is occasionally the case with malignant disease arising in the silent areas. In leuchæmia the anæmia is sometimes explained by reason of the proliferation and extension of the leucopoietic tissue within the bone marrow, to the relative exclusion of erythropoietic tissue. But the anæmia which accompanies leuchæmia, or which develops at some phase of the disease, cannot always be explained on this facile hypothesis. Examination of the marrow shows not erythropoietic hypoplasia, but a definite hyperplasia which gives the pink tinge to the macroscopic specimen, though this hyperplasia is impotent as in the case of most diseases arising from malignant processes. Here, then, is a feature of leuchæmia in common with orthodox malignant disease. The same applies also to cachexia, which is common to malignant disease and leuchæmia, and which in leuchæmia arises even though the cells do not invade and destroy tissue as with orthodox malignant disease. Those who argue against the malignant nature of leuchæmia have to give an explanation of these features.

### LEUCHÆMIC AND ALEUCHÆMIC TYPES

We recognize hæmatological types which we designate as leuchæmic or aleuchæmic according to the state of the peripheral blood; but these do not greatly differ in clinical features, course or prognosis, save that in the aleuchæmic case the lymph nodes, spleen or liver may not be so grossly enlarged at the onset. This aspect, that the disease may be leuchæmic or aleuchæmic, is accepted as a commonplace, a mere variation in manifestations; it has not given rise to argument and serious discussion. Yet the fact that the two types exist suggests the involvement of two different processes which on rational grounds should be physiologically interlocked. I refer, of course, first to the process of maturation, and secondly to the process of release into the circulation. We are woefully ignorant of the nature of any materials which we could confidently call maturation factors—factors, say, which cause the myeloblast to mature to the granulocyte—and we know even less of the forces which regulate the release of granulocytes into the circulation or which restrain the myeloblast from being so released. It is quite certain that a mere *vis a tergo*, by pressure from growing cells, is not the governing factor. In frank leuchæmia there appears to be inhibition or retardation of maturation in whole, or in part, together with lack of control over release, in that cells, including primitive cells, flood the circulation. In the aleuchæmic forms, lack of maturation is a prominent feature; but the release mechanism appears to be largely intact, in that the numerous primitive forms may not reach the circulation in any but small numbers.

When one examines these points, it is abundantly clear that we know little or nothing of the physiology of leucopoiesis, and I am sure that we shall not understand leuchæmia properly until some of these basic physiological facts have been determined. If half the time and energy which have been devoted to the devising and exploiting of empirical remedies for the treatment of the disease had been expended on basic physiology and pathology, we might well be much nearer to a rational treatment. One may mention in passing that the clear recognition of aleuchæmic types as the result of sternal puncture technique has revealed how much more common the disease is than was at one time supposed. There has been a parallel decline in the number of cases diagnosed as aplastic anæmia, which in its primary form must now be considered to be extremely rare. There is no doubt that many cases of aleuchæmic leuchæmia were previously misdiagnosed as aplastic anæmia. But this is not sufficient to account for the remarkable increase in the incidence of leuchæmia during the past twenty years, which has been brought out in many statistical studies, and which has been very evident in my own practice, even when we discount the fact that anyone with a hæmatological reputation tends to collect cases of this disease. I have been even more impressed by the increase in the monocytic types, whether of Schilling or Naegeli, but particularly the latter.

### PATHOLOGICAL CONTRASTS BETWEEN LEUCHÆMIA AND MALIGNANT DISEASE

Let me now comment on one or two points of factual pathology and morbid anatomy which, again, are well known, but which are accepted as ordinary and inevitable, and which have not

given rise to the thought or discussion which they merit. With the fully developed leuchæmic process, all the tissues of the body become infiltrated with leucocytes, and in some sites these may undergo proliferation. It is this feature which gives rise to the remarkable protean clinical manifestations of the disease, so that a leuchæmic patient may present at almost any of the special departments of a large hospital. I need hardly elaborate this point—one thinks of the neurological lesions, Ménière's syndrome, dermatological manifestations, dental trouble, visual changes, bone and joint symptoms sometimes resembling acute rheumatism, hæmaturia, purpura, abdominal tumour, bronchitis and what you will. The leuchæmic infiltration may be gross, thus accounting for pressure symptoms, or may be moderate, or may be so minimal, in some aleuchæmic sclerotic states, that pathologists argue even about the diagnosis. I would remind you, too, that the cells have a characteristic distribution in the liver according to the type of leuchæmia. We recognize the portal distribution in lymphatic leuchæmia, the intercellular and intrasinusoidal distribution in the myeloid type, and the subcapsular collections in the monocytic form. In no other malignant disease, in which the malignant cells are distributed by the blood-stream, does this peculiarly constant deposition and distribution occur. Furthermore, as an additional argument against the malignant nature of the cells themselves, we must acknowledge that the infiltrating cells do not multiply freely and do not invade and destroy the surrounding tissue, nor do they give rise to a stromal reaction. The leuchæmic cells may multiply in certain sites, but the nature of the multiplication suggests a comfortable symbiosis rather than a malignant invasion. It should be noted also that the most characteristic sites for leuchæmic infiltration are the organs in which fœtal blood production occurs—the spleen, the liver and the kidney; organs in which sinusoidal spaces are abundant.

Yet the rule about lack of invasive and destructive qualities is not absolute. The rare chloromatous tumour invades and destroys. The lymphosarcomatous tumour, the cells of which are histologically indistinguishable from the cells of lymphatic leuchæmia, and a disease which sometimes terminates with a blood picture resembling lymphatic leuchæmia, both destroys tissue and gives rise to invasive and destructive metastases.

The issue is not, therefore, a simple one, and any argument against a malignant theory for leuchæmia has to explain the rarer types of leuchæmia which have characteristic malignant features.

### *Nature of the Leuchæmic Cell*

There is convincing evidence, from cultural experiments, that the leuchæmic cell *per se* is not malignant. In the first place, it has long been established that leuchæmic cells develop into normal cells when cultivated *in vitro*; but an additional fact, not so frequently quoted, is that leuchæmic cells *in vitro* proliferate at the same rate as normal cells. Furthermore, it has been very apparent from work in my own department that primitive cells, which in the in-vivo conditions within the body of a leuchæmic patient have been unable to mature, will nevertheless do so quite readily *in vitro*. And even more: these primitive leuchæmic cells, when examined by in-vitro culture, present several points of interest. They may mature into granulocytes. On the other hand, if the culture is contaminated with some innocuous bacterium, the cells can differentiate into macrophages which ingest the bacteria. It is difficult, if not impossible, to quote an example of an orthodox malignant cell which retains its capacity for differentiating into a highly specialized type of non-malignant cell, given the appropriate stimulus. Winston Churchill once said, when speaking of history in relation to current events: "The further one looks back the more one can look forward." So also with medicine, sometimes to our humiliation. It has been forgotten, or at least it has made no impression on current thought, that nigh on a quarter of a century ago certain observers (Timofejewsky and Benewolenskaja, 1927) grew leuchæmic myeloblasts in culture and found that they differentiated into polyblasts in the presence of tubercle bacilli, whereas cultures which contained no tubercle bacilli did not show this cellular differentiation. These observers went so far as to hold that the polyblasts found in cultures infected by tubercle bacilli were rapidly transformed into epithelioid cells and giant cells—indeed a most remarkable capacity for differentiation, when one bears in mind that the cells were originally derived from leuchæmic cells.

What other experimental data have we which go to show that leuchæmic cells do not possess malignant characteristics? Malignant cells when injected into the anterior chamber of the rabbit's eye proliferate readily—so readily that this manœuvre can almost be applied as a criterion of malignancy (Greene, 1948). Yet leuchæmic cells when so implanted neither multiply nor survive (Hoogstratten, 1949).

It would appear, therefore, that the fundamental defect in leuchæmia does not lie with the cells themselves, which, given an altered environment, display all the properties of normal cells in respect of maturation, rate of proliferation and above all capacity to differentiate in response to appropriate stimuli.

### *Environmental Factors*

What, then, can we learn about what we may call the leuchæmic environment? We can obtain hints from the experimental field, from clinical observation and from straightforward post-mortem examination. In the experimental field, if we use first of all the rather questionably comparable type of transmissible mouse leuchæmia, it is well known that certain types of the disease can be transmitted only to animals that have been previously irradiated. One deduces that the soil must be altered before the seed can be implanted. A more reliable lesson can be learned from the human leuchæmic subject. We are all familiar with the leuchæmic nodule of the skin which is sometimes a prominent feature in an individual case. A leuchæmic nodule can readily be produced in the skin of a leuchæmic patient by the subcutaneous injection of a small quantity of normal marrow; the normal cells proliferate and undergo mitosis. If, however, leuchæmic marrow is implanted beneath the skin of a non-leuchæmic person, no nodule is formed (Hoogstratten, 1949). From this we deduce that normal cells placed in a leuchæmic environment behave like leuchæmic cells, whilst the reverse is also true. From the clinical field we can obtain several sidelights. In the first place, leuchæmic blood has several times been transfused in considerable volume, either by accident or by design, in cases of agranulocytosis (Whitby and Britton, 1953); yet in no case has the disease become implanted in the recipient, whilst the transfused leuchæmic cells have behaved as normal cells. Yet how easily do orthodox malignant cells become implanted in the site of excision! We have also other isolated clinical observations, the significance of which has never been seriously discussed; they have merely been recorded in facts. I think first of what is well known to those who see many patients with leuchæmia—namely, that the intrusion of an acute infective stimulus, such as a carbuncle, frequently causes a rapid remission in a case of myeloid leuchæmia, and this reaction is so specific that the onset of glandular fever has been reported as causing a remission of monocytic leuchæmia. The sudden application of a dominating stimulus, an infection, may immediately cause the marrow to revert to normal. This never occurs in malignant disease, though I would remind you of the old-fashioned treatment of sarcomata with Coley's fluid. Does this not all mean that if you alter the environment the process of blood formation can revert to normal? I cannot think of examples of this occurring in orthodox malignant disease other than the doubtful effect of Coley's fluid. The same applies to spontaneous remissions, which all must acknowledge as a clinical fact. What has happened? It must be that the environment has altered, that whatever metabolic fault or deficiency is responsible for the disease has shown a flicker of return to normality. On the other hand, spontaneous remission in orthodox malignant disease is excessively rare. It is perhaps significant that in spontaneous mouse leuchæmia the lymph nodes show an altered metabolism before there is any morphological evidence of leuchæmia (Victor and Potter, 1935).

We should also take note of the clinical fact that when leuchæmia is manifested it appears to arise in all places at once. No post-mortem examination has ever revealed a local leuchæmic marrow lesion. The hyperplasia of leuchæmia is orderly and uniform, and proceeds along the lines of physiological expansion as with an infection, though at a more primitive level. Orthodox malignant processes, on the other hand, begin as a local tumour which afterwards disseminates. With leuchæmia, so far as we can discover, there is no question of a local marrow growth spreading gradually—all marrow, whatever bone is examined, shows that the leuchæmic process arises simultaneously. This surely is unique so far as malignant conditions are concerned, and must cast some doubt either on the malignant nature of leuchæmia, or on any other view than that malignant disease *per se* arises at least in part from a metabolic or environmental fault.

## DEDUCTIONS FROM TREATMENT

Let me turn now to the treatment of leuchæmia and make deductions or suggestions from that aspect of the disease. In the first place I have already drawn your attention to the difference between aleuchæmic and leuchæmic leuchæmia. In the first named the mechanism of maturation is disturbed, but not the release mechanism; in the second, both are involved. When the release mechanism is intact, as in the aleuchæmic types, we are often unable to use some of our empirical remedies such as X rays, urethane, nitrogen mustards

or other antimitotics, though I am not myself averse to employing arsenic in small doses. But when the release mechanism is entirely uncontrolled, as in chronic myeloid leuchæmia with an extremely high count and a very large spleen, then the disease is superficially amenable to the antimitotic empirical remedies. Such cases have the best prognosis in relation to duration of life, likelihood of remissions and response to treatment. There is, therefore, more than an academic difference between leuchæmic and aleuchæmic leuchæmia.

Let me next remind you of a rediscovered fact—the effect of transfusion of normal blood in the case of acute leuchæmia, which *per se* has always been regarded as rampantly fatal. So long ago as 1924 Sabin *et alii* recorded that the transfusion of normal blood to leuchæmic patients appeared to influence the maturation of primitive cells so that the blood picture reverted to normal. Transfusion is now an established and recognized method of treating the acute leuchæmias (Bernard and Bessis, 1948), and one authority (Dreyfus, 1948), in reviewing the literature of acute leuchæmia, concluded that whenever a spontaneous remission is recorded it has been in association with transfusion therapy. The same result can be achieved with a transfusion of fresh plasma, though not with the reconstituted dried product. Is there, therefore, some inhibitory factor in the blood and tissue fluids which prevents the development of leuchæmia ? Or does leuchæmic blood lack some factor without which the dread disease develops ? Is the disease, therefore, no more than an exhausting impotent hyperplasia rather than a true malignant process ? If this last was to be so, then the principles of modern treatment, which, superficially, are designed to inhibit mitosis rather than remedy a deficiency, must be fundamentally wrong. The effect of transfusion in acute cases has to be borne in mind by those who undertake to assess the efficacy of the various new remedies that are under trial. In acute cases it is usually difficult to preserve life long enough for a fair trial of the remedy without also giving a transfusion. It is therefore difficult to say whether any beneficial effect is due to the remedy as distinct from the transfusion. Here, also, in this response to transfusion, is another divergence from orthodox malignant disease, for cancerous processes do not remit after simple transfusion. Here, too, is the germ of hope for the future ; for if this relatively natural and exceedingly conservative form of treatment can induce a remission, what is it that is contained in normal blood or plasma, and that is apparently missing from leuchæmic blood, which brings about this remarkable remission—a remission so remarkable that even the marrow, in my experience, may revert almost to normal ? When the factor in normal blood which induces this remission can be positively identified, we should be on the threshold, if not within the door, of a method of treatment far more realistic and rational than antimitotic measures or assaults with such substances as variants of mustard gas or X rays. These last, to me, appear to be aimed mainly at a symptom and not at a cause.

The same applies to the steroid hormones which with considerable constancy can induce one or more remissions in the acute lymphoblastic leuchæmias, especially in childhood. This remarkable effect induced by a natural substance and not by a cellular insult must be regarded as indicating a step forward in fundamental understanding, and as suggesting that there is more than one factor—metabolic, mitotic, environmental, endocrine and so on—which becomes uncoordinated, disturbed and disintegrated with the inception of leuchæmia.

This opinion leads me naturally to pass on to the modern methods of treatment and to comment on these in the light of what I have already said.

Many remedies are employed. None are more than temporarily effective, whilst some are frankly dangerous in certain forms of the disease.

Arsenic, which was used long before the introduction of X rays and more modern methods, has still a place in treatment. It is possible to maintain a remission in chronic myeloid leuchæmia with arsenic alone, and I still regard this substance as the most suitable for the treatment of leuchæmia in a pregnant woman. One action of arsenic is probably the inhibition of mitosis, and this is true also of colchicine, urethane, X rays, nitrogen mustards, the pterins, myleran, TEM, 6 mercaptopurin and so on. I do not propose to go into detail about these different forms of treatment, but only to remark that, on account of the reduced incidence of unpleasant effects, I have come to prefer methods other than the use of X rays for the treatment and maintenance of patients with chronic leuchæmia, except when there is a large local tumour which is causing pressure symptoms. Rather do I want to comment on points which are relevant to the main theme of my remarks. It has long been known that purine metabolism is grossly disturbed in leuchæmia. It is therefore

interesting that colchicine, which has such a remarkable action in gout, the classical example of disturbed purine metabolism, should also exert an effect in leuchæmia. Furthermore, it has been suggested that the action of urethane falls within this category, in that it may compete with some naturally occurring amine involved in purine synthesis. Treatment based on such hypotheses appears to me to be more rational than treatments, such as the use of X rays, which seem to aim at the destruction of dividing leucocytes. Nevertheless even such drastic treatment may do more than destroy cells. None of us knows what subtle biochemical changes in environment such powerful and incalculable remedies as X rays may bring about, and it may well be that they not only destroy cells, but in so doing liberate a material which has some effect in regulating the leuchæmic process. It has been established that X and $\gamma$ radiations inhibit the synthesis of thymonucleic acid, which is an important constituent of the metaphase of chromosomes, and it seems possible that microchemical studies will show that disturbances of enzyme systems, by relatively simple substances, may be the basis of all derangements of cell metabolism including malignant proliferation. The difficult field of enzyme chemistry would seem to be the one most likely to supply the key to cause and treatment. Studies of this kind should unravel the physiology of leucocyte maturation and release, to which I have referred, and should eventually provide a rational explanation of leuchæmia. However, with new and empirical remedies we should always bear in mind that the mere reduction of a leucocyte count is no more than symptomatic treatment, which may neither induce a clinical remission nor really touch the fundamental cause of the disease. We do not put out a fire by devising methods for abolishing the smoke; we do not cure an infection by abolishing the fever with an antipyretic drug; and we shall not cure leuchæmia by abolishing mitosis. The real problem is to find something which will control mitosis.

## SPECIFIC CAUSAL FACTORS

We must, however, look at the other side of the picture—the evidence for specific causal factors, and also the detection of significant secondary metabolic changes resulting from the leuchæmic process. I have already mentioned the subtle influence of ionizing irradiation on nucleic acid synthesis and cell division, and the whole world is now aware of the unquestionably high incidence of leuchæmia among those irradiated at Hiroshima and Nagasaki. Furthermore, it seems clear that the incidence of leuchæmia among radiologists and others exposed to irradiation hazards is greater than in any other section of the population. There is indeed no doubt that within a certain dose range—single as with an atomic bomb explosion or cumulative and total as with a radiologist—the danger from ionizing irradiations represents one contributory if not causal factor, and it will be remembered that irradiated mice are those which are susceptible to transmissible leuchæmia.

Small wonder, then, that the public has become concerned about the increase in the radioactivity of the planetary atmosphere as the result of atomic explosions, and, indeed, in England a special committee of the Medical Research Council has reported to Parliament on this aspect of modern life.

Irradiation in fact conforms to the usual biological rule in relation to poisons—namely, that small doses stimulate, whereas large doses are lethal when the tolerable level has been exceeded. It is in this last range that radiotherapy is applied.

As to other specific causal factors, it is disappointing that the identification of myeloid and lymphoid factors in urine by the Millers in America has been neither substantiated nor confirmed.

With regard to significant secondary metabolic changes, many interesting sidelights could be mentioned. We are now fully aware of the importance of trace metals, and the presence of cobalt in vitamin $B_{12}$ has underlined this point. It is now known also that the element zinc is a normal constituent of blood and plasma, and that zinc is more concentrated in leucocytes than in any other cells. Cell for cell the leucocytes contain twenty-five times as much zinc as red cells (Hughes and Gibson, 1947), and the interesting feature is that the leucocytes of patients with myelocytic, lymphocytic or monocytic leuchæmia contain only 10% of the normal amount of zinc. But when the patient is treated with X rays or urethane, the falling leucocyte count is accompanied by a rise in zinc content to normal levels. The simple frontal attack suggested by this finding—an attempt to raise the level of zinc and lower the leucocyte count by injection of zinc gluconate—has not been successful (Gibson *et alii*, 1947), whilst it is not clear whether leuchæmic cells are zinc-deficient because they are immature, or whether they are leuchæmic because they are zinc-deficient. But there seems to be a

suggestion from other quarters that there is some redistribution of the trace metals in neoplastic tissues.

## SUMMARY

Let me then marshal some of these rambling facts in a brief summary in order to make my points. First, is leuchæmia a frank malignant disease, as classified by the World Health Organization? In favour of this view, is its inevitably fatal prognosis and its association with the anæmia and cachexia so characteristic of malignant disease, as well as the uncontrollable cell proliferation. But there are features which are unorthodox, including spontaneous remissions, remissions after simple blood transfusion or simple infection, the non-invasive character of the widespread secondary infiltrations, and especially the fact that leuchæmic cells mature *in vitro* to normal cells and at a normal rate, and at the same time preserve their inherent power to differentiate.

Secondly, if leuchæmia is not an orthodox malignant disease, how can we explain its origin and course? Implantation experiments in the skin, the remissions already alluded to, the absence of a local tumour even at the earliest phases and the beneficial action of certain drugs suggest that one factor is an overall environmental alteration brought about by some metabolic defect. In relation to this we obtain occasional glimpses of the workings of metabolic processes which supply pieces of information that may one day fit into the jigsaw of complete understanding. I refer to alterations in purine metabolism, folic acid metabolism and the distribution of trace elements. Thirdly, what of our attempts to treat this disease with such diverse agents as X rays and radioactive isotopes, arsenic, colchicine, nitrogen mustards and folic acid antagonists? Many of these methods are empirical and have no rational basis. Countless time has been spent on empirical treatment and on exploiting remedies which are usually applicable to a neoplasm. I have given you certain criticisms of the neoplastic theory which require to be seriously considered, especially when almost nothing is known of the physiology of leucopoiesis and its normal regulating mechanism. It is very clear that the nature of the leuchæmic process must be definitely established before a logical and rational approach to therapy can be instituted.

It seems likely that the disease itself will be found to arise from a combination of factors involving probably the subtle enzyme systems concerned with cell metabolism and cell division, and not, as yet, to the exclusion of a contributory infection in parallel with the findings in animals. The malignant influence of ionizing irradiations can be regarded as fully established, and it may well be that detailed and systematic studies of the changes in enzymatic and cellular chemistry which these induce will reveal the fundamental pathology of leuchæmia to which more than one factor seems likely to contribute.

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# MACROGLOBULINÆMIA[1]

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THE application of physico-chemical techniques, such as electrophoresis and ultracentrifugation, to the study of human plasma has resulted in the recognition of various highly abnormal protein components. Such components were first demonstrated in multiple myeloma as unique homogeneous proteins in the globulin area of the electrophoretic separation. They were considered to be characteristic of myeloma and were styled " para proteins " (Apitz, 1940) in the belief that they formed no part of the plasma protein complex of healthy subjects.

Another group of abnormal proteins, first recognized in myeloma by Wintrobe and Buell (1933) and occurring most frequently in this disease, show the property of reversible cold precipitation (hence termed " cryoglobulins "). Cryoglobulins have been subsequently identified in a variety of conditions and may even occur as the only manifestation of disease—" essential cryoglobulinæmia ". Cryoglobulinæmia has been recently reviewed by Barnett, Curtain and Hayes (1956) and by Mackay *et alii* (1956).

Waldenström (1944), utilizing the ultracentrifuge, identified a third species of abnormal plasma protein, which he subsequently (1948) termed a " macroglobulin ", because of its high sedimentation coefficient indicative of a molecular weight in the vicinity of 1,000,000. Some, but not all, macroglobulins become so viscid with fall in temperature that they form a gel and so resemble one type of cryoglobulin. The presence of this protein in plasma (macroglobulinæmia) was found to be associated with a typical clinical syndrome which Waldenström initially considered to be a variant of myeloma.

This paper presents the first recorded case of Waldenström's macroglobulinæmia in Australia. The clinical record, physico-chemical data and certain other observations on this patient, together with a survey of the literature, will be utilized to describe the syndrome of primary macroglobulinæmia. It will be emphasized that this syndrome is distinguishable from multiple myeloma, chronic lymphatic leuchæmia and lymphosarcoma, which it resembles, and is unrelated to certain conditions in which minimal quantities of macroglobulins may coincidentally appear.

[1] Received on June 20, 1956.

[2] Working with the aid of a grant from the National Health and Medical Research Council of Australia.

## REPORT OF A CASE

J.C., a man, aged fifty years, presented in January, 1955, complaining of lassitude of eight months' duration, and of increasing tiredness and giddiness present for several days. Eight months prior to his admission to hospital he had developed pneumonia, which lasted for three weeks, after which he " never picked up ". He became easily tired and had a persistent unproductive cough. He denied bleeding tendencies and hæmorrhagic phenomena, but later in the course of the illness he bruised easily and developed a purpuric rash. For nine days he had had attacks of giddiness on change of posture, his tiredness and exhaustion had become worse, and he had had retro-orbital morning headaches. The past history was not significant. A family history was obtained of " anæmia " in two brothers and two sisters.

Examination of the patient revealed him to be a healthy looking man. Pallor was not obvious. The cardio-vascular system was normal, and his blood pressure was 120 millimetres of mercury, systolic, and 75 millimetres, diastolic. Examination of the fundi revealed no hæmorrhages, and the lungs were normal. The spleen, but not the liver, was enlarged. Bulky, firm, mobile lymph nodes were palpable in the neck and axillæ. Initially the skin showed no petechiæ or bruising. The nervous system was normal. The initial diagnosis was " chronic lymphatic leuchæmia ".

The results of significant investigations were as follows: the hæmoglobin value was 8·5 grammes *per centum*, the erythrocytes numbered 4,000,000 per cubic millimetre, and the colour index was 0·72; the leucocytes numbered 20,000 per cubic millimetre, 78% being lymphocytes and 16% mature neutrophile cells; the lymphocytes varied in size, some having quite copious and slightly basophilic cytoplasm (Figure I). The reticulocytes were less than 1%, and platelets numbered 158,000 per cubic millimetre. The pathologist, Dr. D. C. Cowling, stated that the blood picture was not characteristic of lymphatic leuchæmia. Sternal myelography revealed a lymphocytosis of 80%; the lymphocytes were mainly small, many appearing to be devoid of cytoplasm—" naked nuclei " (Figure II). An X-ray examination of the chest revealed prominent hilar shadows, widening of the upper mediastinum probably due to paratracheal nodes, and a small left pleural effusion.

In October, 1955, the liver, spleen and lymph nodes were enlarged. The presence of pancytopenia was reflected by obstinate infection, recurrent bleeding from superficial wounds, skin petechiæ and bruising. The blood findings were as follows: the hæmoglobin

value was 6·2 grammes *per centum*; the leucocytes numbered 55,000 per cubic millimetre, 92·5% being lymphocytes, of which 6% were smear cells; reticulocytes numbered 3·5% and platelets 40,000, and subsequently 17,000 per cubic millimetre; occasional plasma cells were seen. The sternal myelogram was unchanged, except that rouleaux formation was noted.

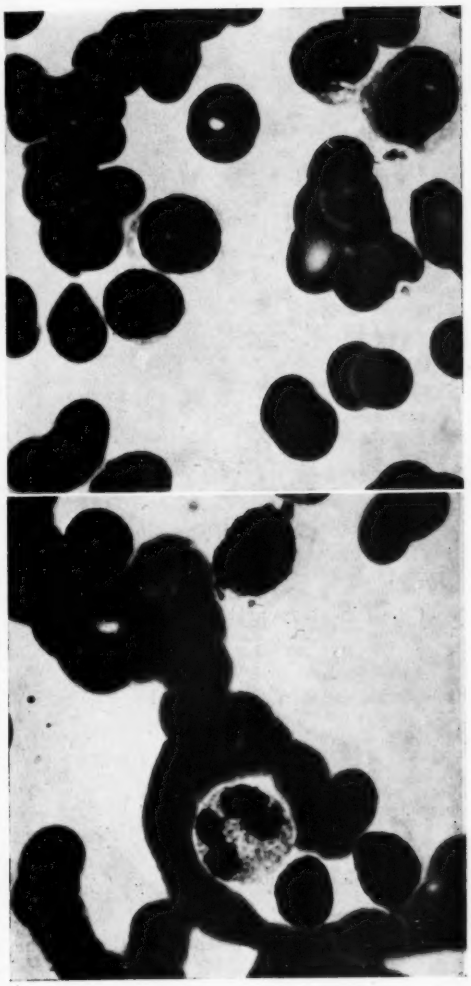

FIGURE I

Peripheral blood, two views (×580), showing atypical lymphocytes. There is rouleaux formation of erythrocytes

The formol gel test gave a positive result. Paper electrophoresis of serum revealed a myeloma-like component (Figure III) comprising 56% of the total serum protein, which was 9·6 grammes *per centum*. Bence-Jones urinary protein was present in small amount. Although a skeletal survey gave negative results, a diagnosis of multiple myeloma was entertained.

However, the presence of lymphocytic cells in the bone marrow and the myeloma-like electrophorogram suggested that the serum be examined by the ultracentrifuge, and this revealed a macroglobulin component in high concentration (Figure V).

Treatment consisted of the administration of triethylene melamine (TEM), thiotriethylene phosphoramide (thioTEPA), cortisone and ACTH, and frequent blood transfusions; TEM therapy was followed by the hypersplenic phase described by Bolton and Bean (1954). Finally, splenectomy was performed in October, 1955, with considerable benefit. Histological examination revealed diffuse infiltration of the splenic pulp with lymphocytic cells; the follicular structure was preserved.

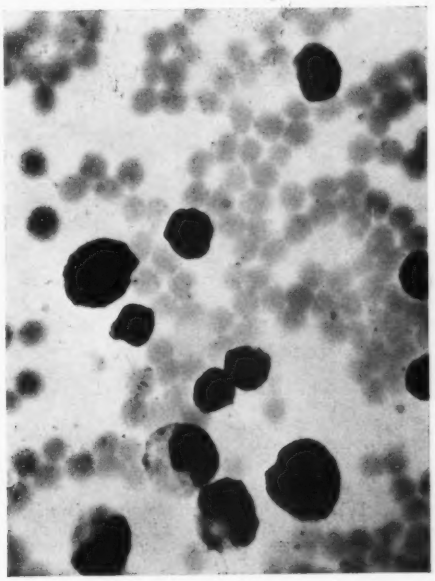

FIGURE II

Bone marrow (×580); diffuse infiltration with lymphocytic cells, with loss of cytoplasm

In March, 1956, a further relapse occurred, a pulmonary infection being followed by a massive left hæmorrhagic pleural effusion. It is of interest that the macroglobulin was found in this effusion (Figure III).

The following laboratory investigations were performed relevant to the diagnosis of macroglobulinæmia: (i) Refrigeration of serum for seven days at 4° C. revealed no evidence of cryoglobulinæmia. (ii) The Sia test for macroglobulinæmia (a dense cloudiness on the addition of a drop of serum to distilled water) produced a positive result. (iii) A serum glucosamine estimation, performed by Miss S. Weiden, showed a considerable elevation to 260 milligrammes per 100 millilitres; the normal range is 70 to 120 milligrammes per 100 millilitres (Weiden, 1956). In a second recent case of macroglobulinæmia also there was an elevated level (286 milligrammes per 100 millilitres). (iv) Electrophoretic analyses gave the following results: (*a*) The paper electrophorogram revealed a discrete abnormal component in high concentration (5·9

grammes *per centum*) in the γ globulin area, present in both serum and pleural fluid (Figure III). (*b*) Free electrophoresis showed a spike macroglobulin component, having a mobility of $-0{\cdot}90 \frac{\text{cm.}^2}{\text{volt sec.}} \times 10^{-5}$ ultracentrifuge at 830 revolutions per minute for sixty minutes; serum was diluted to one in five in 0·2M saline.) (vi) The plasma was shown by Dr. D. Metcalf, of this Institute, to contain a factor stimulating lymphocytosis in baby mice (Metcalf, 1956).

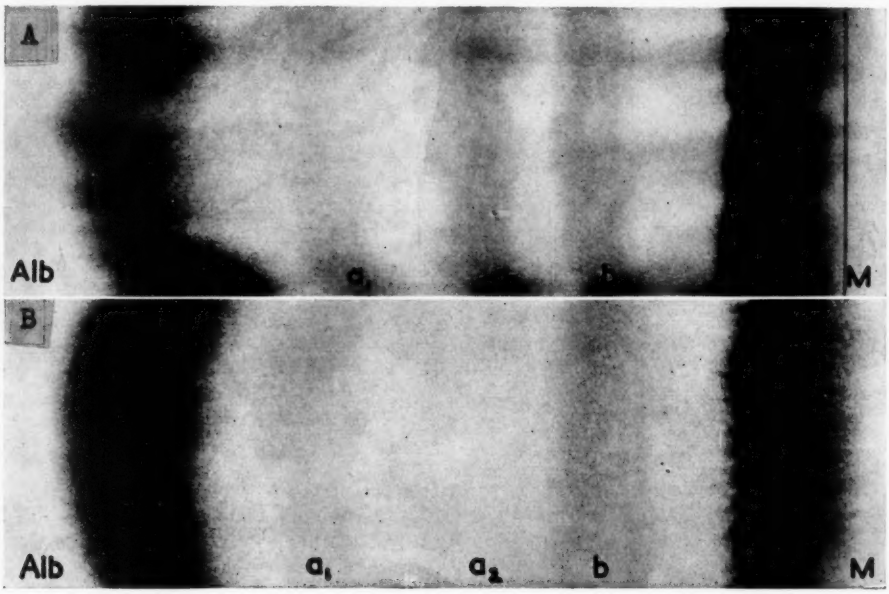


FIGURE III
Filter paper electrophoresis of serum (A) and pleural fluid (B). Discrete macroglobulin component (M) in γ globulin area

(Figure IV). (v) Ultracentrifuge analysis of serum revealed an abnormal component in high concentration, with a sedimentation coefficient of 12·9 S units, and

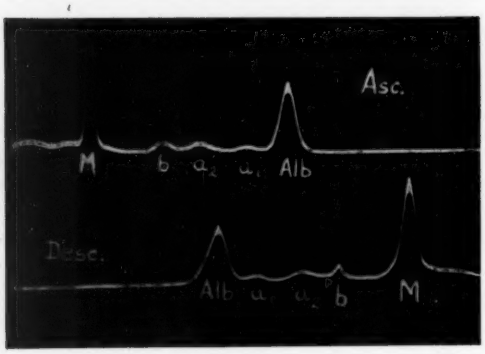


FIGURE IV
Moving boundary electrophoresis of serum. "M" designates macroglobulin component

a small amount of a heavier, faster-sedimenting (17·5 S) component. (Analyses were carried out by Mr. E. F. Woods, who used a Phwye air turbine

*Summary*

An elderly man complained of lassitude for eight months after pneumonia, and subsequently a bruising tendency, with clinical findings of hepatosplenomegaly and lymphadenopathy. Blood and marrow examination suggested a diagnosis of chronic lymphatic leuchæmia. Macroglobulins were demonstrated in serum.

## DISCUSSION

### *The Clinical Syndrome of Macroglobulinæmia*

It will be appreciated that the case history of the patient described in this report exemplifies most of the features of macroglobulinæmia. The patients are usually elderly men, with initial symptoms of weakness, lassitude and dyspnœa of very gradual onset, and a pronounced bleeding tendency, manifested by mucosal hæmorrhages (particularly in the naso-pharynx, mouth and bowel), retinal hæmorrhages, and more rarely petechiæ, purpura or bruising. These are all manifestations of anæmia and thrombocytopenia. Symptoms suggestive of peripheral vascular stasis (Raynaud's phenomenon and peripheral

gangrene) rarely occur, even when the macroglobulin is also a cryoglobulin.

Examination reveals signs of anæmia with or without cachexia and weight loss, slight œdema, and frequently evidence of extravasation of blood into mucosal membranes or skin. Hepatosplenomegaly, with a moderate degree of painless lymph node enlargement, occurs in about two-thirds of reported cases. Laboratory investigations show normochromic anæmia, neutropenia with relative or absolute lymphocytosis (and possibly monocytosis), a normal or reduced platelet count, often a prolonged bleeding time and a normal clotting time. Increased hæmolysis may occur; a "hypersplenic" phase followed TEM treatment in the present case. Punched-out bone lesions, characteristic of multiple myeloma, are absent, although there may be general osteoporosis. Bone marrow examination can be almost diagnostic in showing a large number of small lymphocytic cells, many with scanty or no cytoplasm. These cells are usually, but not always, readily distinguishable from plasma cells (*vide infra*). Recurrent infections, particularly pulmonary, may take place, and are attributable to neutropenia and to low levels of normal γ globulin with resulting immunological failure, similar to that occurring in multiple myeloma (Zinneman and Hall, 1954). Sjögren's syndrome may occasionally complicate macroglobulinæmia (Wuhrmann, 1952).

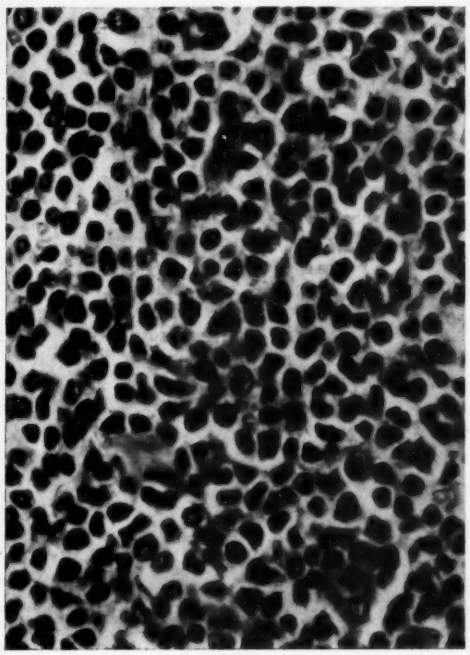

FIGURE V

Excised lymph node; diffuse infiltration of node by lymphocytic reticulum cells. (From patient R.E., under care of Professor R. J. Lovell)

The presence of an abnormal protein in serum is reflected by the following: elevated serum globulin levels (up to 10 grammes *per centum*), a consistently and greatly elevated erythrocyte sedimentation rate, increased serum viscosity often with formation of a gel on cooling which "melts" on warming, a positive response to the formol gel test, and rouleaux formation in blood films. The Sia reaction, developed over thirty years ago in Asia as a test for kala-azar, is a suggestive but not always conclusive test for macroglobulins (Waldenström, 1952; Milhaud and Goldberg, 1950; Mackay *et alii*, 1956). Electrophoresis on filter paper shows a discrete myeloma-like component in high concentration (a spike by free electrophoresis), in the β-γ globulin area of the pattern. The diagnosis is confirmed by ultracentrifuge demonstration of serum globulins of high molecular weight, or by immunological analyses (*vide infra*).

The endothelial barriers of the body are permeable to both cryoglobulins and macroglobulins, the latter having been demonstrated in cantharides blisters (Wuhrmann, 1952), in cerebro-spinal fluid (Mackay *et alii*, 1956), and in the present instance, in a pleural effusion.

The present case conforms to the recognized clinical picture in most respects, except for the initial lack of hæmorrhagic phenomena. A peripheral blood picture, closely simulating chronic lymphatic leuchæmia throughout the illness, is exceptional in macroglobulinæmia, despite the resemblance of the two diseases.

The features of 25 proven cases of macroglobulinæmia reported in the literature are recorded in Table II as a basis for comparison with the present case report.

### *The Differential Diagnosis*

The presence of macroglobulinæmia should be considered in obscure reticulo-endothelial disorders (particularly atypical cases of "lymphatic leuchæmia", "lymphosarcoma" or "multiple myeloma") showing the following features: resistant anæmia, hæmorrhagic phenomena (mucosal bleeding, purpura or bruising), pancytopenia, hæmolytic anæmia, a very high

erythrocyte sedimentation rate, and unexplained hyperglobulinæmia.

Macroglobulinæmia closely resembles lymphatic leuchæmia and lymphosarcoma in its course, clinical behaviour and marrow picture, but is distinguished by the presence in serum of an abnormal species of globulin which is not produced in lymphatic leuchæmia. Cases of "lymphatic leuchæmia" and "lymphosarcoma" with cryoglobulinæmia, reviewed by Mackay *et alii* (1956), may in fact have been examples of Waldenström's syndrome, as well as other cases of chronic lymphatic leuchæmia (usually aleuchæmic) and "lymphatic leucosis" in which abnormal globulins were present (Wuhrmann *et alii*, 1950; Rundles *et alii*, 1955; Rappaport and Johnson, 1955; Batty Shaw, 1956).

TABLE I

*Explanation of Clinical Manifestations in Macroglobulinæmia*

| | |
|---|---|
| Anæmia .. .. | Bleeding tendency<br>Hæmolysis |
| Pancytopenia .. .. | Marrow depression by cell infiltration.<br>Surface damage to blood cells by paraprotein |
| Mucosal hæmorrhages and purpura | Platelet deficiency<br>Interference with platelet function (Braunsteiner *et alii*, 1954)<br>Adsorption of coagulation factors upon the macroglobulin; interaction of macromolecules with fibrinogen; increased heparin production by mast cells (Tischendorf and Hartmann, 1950)<br>Damage to capillary wall by abnormal proteins |
| Cold sensitivity and Raynaud's phenomenon | Cryoglobulinæmia |
| Recurrent infections | Failure of antibody formation possibly due to competitive synthesis of abnormal protein<br>Neutropenia |
| Hepatosplenomegaly<br>Lymph node enlargement | Infiltration with lymphocytoid cells |
| Increased erythrocyte sedimentation rate, rouleaux formation in blood films, autoagglutination of red cells | Abnormal protein present in blood |

Evidence in favour of a differentiation from multiple myeloma includes absence of bone lesions, the rarity of Bence-Jones protein, the presence of high molecular weight globulins in serum (rarely, if ever, found in myeloma), the characteristic morphology of the marrow cell, and the lack of cross reaction of anti-macroglobulin rabbit sera with myeloma proteins.

Since filter paper electrophoresis of serum is now a common clinical laboratory procedure, it is pertinent that the combination of a myeloma-protein component with a bone marrow free of plasma cells is highly suggestive of macroglobulinæmia.

There has been considerable comment in the European literature on various types of hyperglobulinæmia; apart from well-recognized causes, including multiple myeloma and macroglobulinæmia, two other subgroups have been described by Waldenström (1948, 1952): (*a*) "Idiopathic essential hyperglobulinæmia", which is probably not a distinct entity; (*b*) "purpura hyperglobulinemica", which is characterized by a γ globulin increase (electrophoretically a broad hump rather than a spike), purpuric manifestations on the skin rather than mucosal bleeding, and a relatively benign course. Macroglobulins are not demonstrable. This condition may comprise several disease entities, including *lupus erythematosus*.

Macroglobulins are present in serum in health, comprising 1% to 3% of total serum protein, and in low concentration in nephrosis and liver disease (Jahnke and Scholtan, 1953), in congenital syphilis (Willi *et alii*, 1954), and in *lupus erythematosus* (Waldenström, 1952), without the clinical features of Waldenström's macroglobulinæmia. The macroglobulins present in health and in these diseases differ ultracentrifugally and electrophoretically (spike components are lacking), and they do not react immunologically with antisera prepared from Waldenström - type macroglobulins (Kanzow, Scholtan and Muting, 1955). Primary macroglobulinæmia is thus an entity unrelated to the usually low-grade increase of macroglobulins associated with these chronic diseases.

### *The Pathology of Macroglobulinæmia*

Systematized lymphoid hyperplasia throughout the reticuloendothelial system is the characteristic histological finding. The appearances are suggestive of chronic lymphatic leuchæmia or lymphosarcoma. The bone marrow is consistently invaded by cells, variously designated as "lymphoid mononuclear cells", "lymphocyte-like cells" or "lymphocytic elements", although specific differences from true lymphocytes are not described, except for loss of cytoplasm; they comprise from 20% to 90% of total marrow cells. These lymphocytic cells probably manufacture the abnormal globulins (Abrams, Cohen and Meyer, 1949). Other evidence concerning the source of various "paraproteins" is reviewed by Rappaport and Johnson (1955) and by Mackay *et alii* (1956).

The architecture of lymph nodes is obliterated by masses of similar cells, without invasion of

TABLE II

*Clinical, Laboratory and Physicochemical Findings in 25 Cases of Macroglobulinæmia*[1]

| Clinical Findings | Number of Cases | Laboratory Findings | Number of Cases | Physicochemical Data[2] | Number of Cases |
|---|---|---|---|---|---|
| Sex: | | Hæmoglobin value (grammes *per centum*): | | Electrophoresis: | |
| Male | 18 | Under 6 | 4 | Homogeneous component—"spike" | 18 |
| Female | 7 | 6 to 10 | 14 | Diffuse component | 2 |
| Age: | | Over 10 | 5 | Mobility: | |
| Under 50 years | 3 | Leucocyte count (thousands per cubic millimetre): | | β globulin | 4 |
| 50 to 70 years | 18 | Under 6 | 14 | Between β and γ globulin | 8 |
| Over 70 years | 4 | 6 to 10 | 8 | γ globulin | 11 |
| Weakness, lassitude | 22 | Over 10 | 1 | Ultracentrifuge: | |
| Weight loss | 8 | Lymphocytosis: | | S values: | |
| Mucosal bleeding | 19 | Relative | 11 | 16 to 20 | 12 |
| Respiratory infections and pneumonia | 7 | Absolute | 1 | 12 to 16 | 5 |
| Hepatosplenomegaly | 19 | Thrombocytopenia | 6 | Multiple components | 8 |
| Lymph node enlargement | 15 | Prolonged bleeding time | 2 | | |
| Serous effusions | 4 | Hæmolytic phenomena | 1 | | |
| Skeletal survey: | | Lymphocytoid cells in marrow (20% to 90%, often with plasma cell increase also) | 23 | | |
| Osteoporosis | 6 | Erythrocyte sedimentation rate: | | | |
| Myeloma translucencies | 0 | Over 70 per hour | 20 | | |
| Coarse trabeculation | 1 | Over 100 per hour | 16 | | |
| | | Not stated | 4 | | |
| | | Total protein content over 8 grammes *per centum* | 17 | | |
| | | Sia test result positive | 15 | | |
| | | Cryoglobulinæmia | 15 | | |
| | | Elevated serum viscosity | 7 | | |
| | | Bence-Jones proteinuria | 5 | | |
| | | Formol gel test result positive | 8 | | |

[1] From cases reported by Waldenström (1944), Bianchi *et alii* (1949), Dalgaard (1950), Lucey *et alii* (1950), Tischendorf and Hartmann (1950), Schaub (1952, 1953), McFarlane *et alii* (1952), Wilde and Hitzelberger (1954), Kanzow (1954), Pernis *et alii* (1954), Braunsteiner *et alii* (1954), Mandema *et alii* (1955), Mackay *et alii* (1956).

[2] Concentration of macroglobulin ranged from 7% to 75%, usually 10% to 30% of total serum protein content.

the capsule (Figure VI). The spleen and liver may also show lymphocytic infiltration. Increased tissue mast cells are occasionally reported in the bone marrow and lymph nodes (Dalgaard, 1950; Tischendorf and Hartman, 1950; Mandema *et alii*, 1955). Dalgaard (1950) and Mandema (1955) have described cells transitional between lymphocytes and plasma cells, and in several instances, particularly in terminal phases of the disease, lymph nodes and marrow presented a true plasma cell increase.

Schaub (1953) noted the coincidental occurrence of macroglobulinæmia in a single case of uterine carcinoma. Renal deposition of abnormal protein may produce tubular changes, as occurs in multiple myeloma.

### *Prognosis*

The available data suggest that macroglobulinæmia pursues a slower course than multiple myeloma, leuchæmia and other reticuloses; in four cases ending fatally, the disease had been present for three, four, five and ten years respectively. No recoveries or permanent remissions have been recorded, and the eventual fatal outcome is due usually to bone marrow replacement, with pancytopenia associated with severe bleeding and infection. A cerebral hæmorrhage may be the final event.

### *Treatment*

Treatment follows the lines employed for other reticuloses, and is based upon supportive therapy and transfusions. Cortisone and ACTH

TABLE III

*The Differentiation of Macroglobulinæmia from Closely Related Conditions*[1]

| Condition | Focal Bone Lesions | Bence-Jones Proteinuria | Electrophoretic "Spike" Component | High Molecular Weight Globulins | Highly Elevated Serum Glucosamine | Mouse Lymphocytosis Stimulating Factor in Serum |
|---|---|---|---|---|---|---|
| Primary macroglobulinæmia | − | ± | + | + | + | + |
| Multiple myeloma | + | + | + | − | − | − |
| Lymphatic leuchæmia and lymphosarcoma | − | − | − | − | − | + |
| Lymphadenoma | − | − | − | − | + | − |

[1] Traces of Bence-Jones protein were present in only five of 25 reported cases.

may produce temporary remissions, urethane has caused suppression of the paraproteinæmia, and splenectomy, as in the present case, may be of considerable benefit if a hæmolytic element or "hypersplenism" is present; in other cases (Mackay *et alii*, 1956) it has had little effect. Experience with nitrogen mustard, TEM and thioTEPA is scanty; the two last-mentioned drugs induced temporary remissions

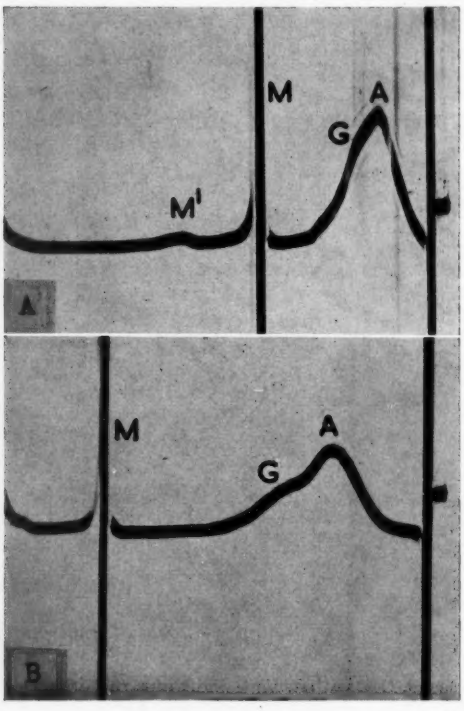


FIGURE VI
Ultracentrifuge analyses of serum; photographs taken after ten minutes (A) and thirty minutes (B). A=albumin, G=γ globulin, M and M′ are macroglobulin components. Such an M peak is seen only in primary macroglobulinæmia

in this patient. Radiotherapy, $P_{32}$, stilbœstrol and diamidine have been tried without much success.

*Physicochemical Properties of Macroglobulins*

Physicochemical studies emphasize the lack of uniformity in the properties of macroglobulins. They migrate electrophoretically with either the β or γ globulins, commonly in the $\gamma_1$ or fibrinogen area, as a homogeneous "spike" component; but broad peaks may also occur (Wilde and Hitzelberger, 1954). In the ultracentrifuge, there are usually one major component in the 18 Svedberg unit range and one or more faster-sedimenting (18 to 28 S) components which appear to result from dissociation reactions involving serum macroglobulins (Pederson, 1949; Petermann and Braunsteiner, 1954). The unusually low 13 S value of the major component in the present case may be related to concentration dependence; a precise S value would require several analyses with extrapolation to infinite protein dilution.

A viscid gel formation commonly occurs when macroglobulin serum is cooled, suggesting cryoglobulinæmia; other types of cryoglobulin formation (flocculum, crystals, two-phase separation) seldom occur.

*The Chemical Structure of Macroglobulins*

Aminoacid analyses (Pernis, Wuhrmann and Wunderly, 1954) showed the composition of four macroglobulins to be different; however, a general pattern existed, which was intermediate between that of normal β and γ globulins, in accordance with their usual electrophoretic mobility. Lysine and proline were both present in lower amount than in normal globulins. Mandema (1955) also found a low proline content in a macroglobulin, although the lysine content was normal. The analyses by MacFarlane *et alii* (1953) yielded no particular findings. There is no lack of methionine as in Bence-Jones protein. End group analyses of a macroglobulin (Putnam, 1955) showed a lack of N-terminal aspartic acid, in contrast to normal γ globulin.

*Immunological Properties of Macroglobulins*

The concept that the aberrant "paraproteins" of myeloma and macroglobulinæmia have no counterpart in the normal plasma globulin spectrum is borne out by immunological studies. Slater, Ward and Kunkel (1955), in a study of 21 myeloma sera by precipitin reactions, found that all the abnormal myeloma proteins were immunologically distinct, both from each other and from available globulin fractions of normal serum. Macroglobulins show analogous immunological behaviour. Anti-macroglobulin rabbit sera were prepared by Habich (1953), and in a study of 16 cases of macroglobulinæmia by precipitin testing, he demonstrated (i) that each macroglobulin was antigenically individual and specific, and differed from patient to patient—that is, like myeloma proteins, different macroglobulins apparently have a different antigenic (and possibly

chemical) structure; (ii) that in the majority of cases there was a group antigen specific for macroglobulinæmia sera alone, and absent from the serum in health and in various other diseases, including leuchæmia and myeloma.

In a recent study, Kanzow, Scholtan and Muting (1955) confirmed Habich's finding of specific antigens in macroglobulinæmia sera, and demonstrated that such antigens were in the macroglobulin component. The macroglobulins present in sera of diseases other than Waldenström's syndrome (nephrotic syndrome, cirrhosis) did not precipitate in significant titre with antimacroglobulin rabbit serum. Specific precipitation also failed to occur with multiple myeloma sera.

Thus, whereas myeloma proteins are related to, but not identical with, normal serum globulins, abnormal macroglobulins appear immunologically to be quite unrelated to any normal serum globulin, including (probably) the small macroglobulin component normally present in serum, at least as far as rabbit antibody testing is concerned.

### *The Individuality of Primary Macroglobulinæmia*

Of particular interest, and not previously described, is the finding by Dr. D. Metcalf of a mouse lymphocytosis stimulating factor (in weak titre) in the serum of this patient—hitherto found only in chronic lymphatic leuchæmia, lymphosarcoma and myelofibrosis, and absent in multiple myeloma (Metcalf, 1956). Secondly, highly elevated (over 260 milligrammes per 100 millilitres) serum glucosamine levels, present in this patient, are found only in certain of the reticuloses, notably lymphadenoma, but not in chronic lymphatic leuchæmia. Although these findings favour the concept that primary macroglobulinæmia is a disease entity of a "reticulosis" ("lymphoma") type, and not a variant of multiple myeloma, it is still uncertain how precisely this syndrome can be differentiated from the group of lymphocytic neoplasias.

### *The Relationship of Macroglobulinæmia to Somatic Mutation*

Macroglobulinæmia may eventually prove to be a condition or group of conditions of high significance for the development of the somatic mutation theory of the origin of the wide range of neoplastic states seen in elderly patients.

It is a cardinal point of the theory that only those mutations which result in an increased growth potential of the affected cell can have any significant influence. This may be similarly expressed by stating that only if the mutation results in the loss of some structure or function by which general control over growth is exercised, will the descendants of the mutated cell become sufficiently numerous to produce clinically or chemically detectable effects. The commonest effects will, of course, be those resulting directly from malignant growth of the cell descendants in question.

If the somatic mutation hypothesis is correct, and particularly if it is put in the form suggested by Armitage and Doll (1954)—namely, that a sequence of six or seven mutations is needed for the development of overt malignancy—then there should be occasional opportunities for mutations not themselves leading to or toward malignancy, to become manifest because they have occurred in a cell line which has also become malignant.

Macroglobulinæmia is always associated with a gross replacement of the bone marrow by lymphocyte-like cells which are clearly neoplastic, and which in a small proportion of cases give a blood picture hardly distinguishable from chronic lymphatic leuchæmia. Whenever the abnormal proteins from different subjects of macroglobulinæmia have been compared, they have shown sharp individual antigenic differences; thus immunologically (and therefore genetically) the appearance of the capacity to produce macroglobulins in excess cannot be directly related to the changes resulting in gross proliferation of the abnormal lymphocytes. It must represent an independent mutation occurring in the same cell line as that from which the neoplastic cells have sprung, or be due to some wholly unexplained process. Of the known biological processes only independent somatic mutation is capable of covering the facts, including particularly the rarity of the condition, its age incidence, and the heterogeneous serological character of the abnormal protein.

In fact it may well be claimed that the existence of this syndrome provides one of the strongest pieces of evidence for the correctness of the somatic mutation hypothesis of neoplastic disease.

### SUMMARY

A case of primary (Waldenström's) macroglobulinæmia presented with a clinical picture simulating chronic lymphatic leuchæmia.

The chief clinical features were weakness, dyspnœa, bleeding manifestations, lymphadenopathy, hepatosplenomegaly, and bone marrow infiltration with small lymphocytic cells of a malignant type.

An electrophoretically homogeneous γ globulin component was present in serum; the sedimentation coefficient, by ultracentrifugal analysis, was that of an abnormally high molecular weight globulin or "macroglobulin".

Evidence is provided for the concept that macroglobulinæmia is an individual disease entity, which resembles, but is not a variant of, multiple myeloma, chronic lymphatic leuchæmia and lymphosarcoma.

The synthesis of macroglobulins by malignant lymphocytic cells is considered to be a result of a somatic mutation in the same cell line as that from which these cells have originated; this provides strong evidence for the correctness of the somatic mutation hypothesis of cancer.

## ACKNOWLEDGEMENTS

I am indebted to Sir Macfarlane Burnet for the development of the concept of the relationship of macroglobulinæmia to the somatic mutation theory of cancer.

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# THE PATHOLOGY OF ACUTE BRONCHIOLITIS—A STUDY OF ITS EVOLUTION

## PART I: THE EXUDATIVE PHASE[1]

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*Ex parvis saepe magnarum momenta rerum pendent*
—Livy, *History*, Book XXVII, Section 9.

ATTENTION is drawn in this paper to the tardily accepted pathological tenet that acute bronchiolitis is the focal point of inflammatory disease of the lung. That this view is not accepted as axiomatic is due to a neglect of the study of morphological changes in bronchioles. The reason is not far to seek. Examination of the minute anatomy of evolving bronchiolar disease requires specially prepared material, much of which must be serially sectioned and reconstituted to give a three-dimensional picture; technical problems are constantly encountered. Also, many examples of the disease in various stages of its development must be examined in this tedious way to show its evolution, so that the fourth dimension of time is added to give the entire picture.

An attempt has been made by the writer to gain this end and to present a unified account. The observations made indicated the need for a reconsideration of the complex subject of the establishment of the common air-borne infections of the lungs, about which a great deal of information is already available. In this paper, therefore, this information is critically reviewed, and a presentation of the pathological anatomy of these infections follows in which personal observations are interpolated with evidence from the literature.

It is admitted that not all forms of acute bronchiolitis are included; but, by concentrating on those due to the causes seen in practice, it is hoped in these papers to present a composite picture of acute bronchiolitis in its proper perspective within the wider framework of acute pulmonary infections in general.

### MATERIALS AND METHODS

Serial section formed the basis of this investigation. The main observations were made on 27 selected blocks of lung tissue, from each of which 400 to 1200 serial sections were cut at a thickness of 7 μ. This material was culled from over 120 blocks averaging 2·0 by 2·0 by 0·7 centimetres in size and obtained from approximately 70 patients. A few serial sections were cut from all these blocks, and the final selection was made on pathological significance and technical quality. In all, nearly 20,000 sections were cut.

The group of patients from which this material was obtained varied widely in age and in the conditions leading to death.

Fixation presented a major difficulty. Initially, the excised lungs were fixed by endobronchial perfusion of 10% formalin-acetate solution at a minimum of eight hours after death. Post-mortem autolysis of the basement membrane occurs so frequently within this time that detachment of the epithelium rendered observations on epithelial structure of little value. For this reason cadavers were fixed with 10% formalin-acetate solution run into the femoral vein (with the femoral artery opened) within one to two hours of death. A minimum of 20 litres was perfused, the injection being maintained until the femoral artery returned almost clear fluid. The lungs were removed eight or more hours later. No detachment of epithelium was found in this material.

An important advantage of the intravenous method is that the lungs are fixed in their normal anatomical form. Lungs fixed after removal can be over-distended, and they inevitably show the effects of gravitational flattening. Another advantage, particularly pertinent to this investigation, is that the contents of the air passages are left undisturbed. Endobronchial injection of fixative inevitably drives the contents of the larger bronchi towards the periphery, and gives material which is obviously unsuitable for observations on bronchiolar plugs as they existed before death.

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Engel (1947) presented similar objections to endobronchial fixation and simply floated tissue in fixative fluid. However, the problem of delayed fixation remains, and distortion of the terminal air passages is such that observations made by this method are limited in their significance.

After intravenous fixation the pulmonary vessels were generally empty of blood (a technical advantage), and were considerably dilated in comparison with the contracted vessels seen after endobronchial perfusion. Vessel size in both methods was compared with that apparent in pulmonary angiograms, and the intravenous method gave a vessel size more closely related to that seen in the living.

These numerous advantages determined, in the second half of the investigation, the use of lung tissue fixed intravenously *in situ*. Twelve of the 27 extensively sectioned blocks were fixed by this method, and observations on bronchiolar obstruction by fluids were accepted only in material fixed in this way. Permanent obliterative changes can be identified in tissue fixed by any method, but are more readily recognized in tissue fixed by the intravenous route.

Most of the sections were stained with hæmatoxylin and eosin, but as elastic tissue stains are essential to depict the anatomy of damaged bronchioles, considerable use was made of French's modification of Weigert's resorcin-fuchsin stain (Gurr, 1953) combined with unblued hæmalum and tartrazine. Numerous special stains were used, including Alcian blue and periodic acid-Schiff stain for mucin, silver impregnation for reticulum, Gomori's trichrome and van Gieson's stain for collagen, Fitzpatrick's modification of Weigert's Gram stain, and Löffler's methylene blue for bacteria.

Reconstruction of appropriate areas was made from groups of serial sections.

## General Features of Pulmonary Infection

This review is intended to orientate well-accepted observations on pulmonary infections so that the central role of bronchiolitis is indicated. The aim of the review of pathological anatomy that follows (in which appropriate personal observations are included) is to support and extend this concept.

Classification of air-borne infections according to the specific agents—viral, rickettsial, bacterial or fungal—places undue emphasis on the significance of the infecting agent. Most respiratory tract infections are associated with relatively few types of microorganisms; these include viral (and presumed viral) infections on the one hand and, on the other, a limited group of pyogenic bacterial infections.

As there is some confusion about the bacterial group, it is necessary to discuss these organisms so that the terms used subsequently will be clear.

Only a small number of bacterial species, such as group A hæmolytic streptococci, types I and II pneumococci and possibly *Hæmophilus influenzæ*, are readily capable of spread and proliferation in living tissues. Other bacteria multiply and spread mainly on body surfaces; but some of them—for example, *Staphylococcus aureus*—also produce particularly potent specific toxins which diffuse into the tissues, producing changes often leading to necrosis. They may then multiply freely in the dead tissue.

The readily invasive bacteria and the more toxic of the less invasive bacteria constitute a group which is usually described as " virulent " or " pathogenic ". However, many pyogenic pulmonary infections characterized by fever, leucocytosis and purulent sputum (such as most cases of acute bronchitis or bronchiectasis) cannot be shown to be due to organisms of the types mentioned, and " non-specific " organisms such as green streptococci, *Streptococcus fæcalis*, *Escherichia coli*, non-invasive pneumococci and *Neisseria catarrhalis* may be present in abundance. These organisms cannot be termed " non-pathogenic " (or " avirulent "), as they are clearly pathogenic in the circumstances.

For this reason this group will be referred to as " non-specific ", and the so-called " virulent " or " pathogenic " group as " specific ". It is intended to show how, in appropriate conditions, the non-specific group becomes pathogenic.

Both " specific " and " non-specific " bacteria frequently enter the lower part of the respiratory tract. They are commonly present in the naso-pharynx, and there is no reason to suspect that, as Scadding (1952) points out, mucus containing these organisms differs in behaviour for lipiodol which, when placed in the nose during sleep, quickly descends to the lower part of the respiratory tract. Thus, it can be assumed that bacteria repeatedly enter the lower part of the respiratory tract both in inspired air and in descending mucus, and that the establishment of infection by them depends primarily on changes in host physiology.

Changes which reduce the resistance to infection of the body tissues generally, including the lung, play a part; in such conditions as old age, inanition, diabetes or toxæmia from non-pulmonary infection the incidence of

pulmonary infection is increased. However, local changes in function are more important even when, as in the above mentioned states, general reduction of tissue resistance is also present.

Normal lung function depends on the maintenance of patent air passages with healthy walls, and, as secretions and organisms are constantly appearing in the lumina of normal air passages, homœostatic mechanisms obviously exist to prevent air passage obstruction and infection. Failure of these mechanisms in any part of the lungs produces the local bronchial predisposing factors to lung infection. The two prominent factors are obstruction of air passages and damage to their walls by preceding viral infection.

The supreme clinical importance of these factors in the establishment of pulmonary bacterial infection is well recognized (Scadding, 1952; Lefroy, 1955); but there has been little study of pathological anatomy to show the manner in which these factors operate. This aspect is emphasized here, and it is necessary to review the homœostatic mechanisms and to analyse the nature of changes which induce their failure.

## Homœostasis in the Air Passages of the Lung

The pulmonary air passages are constantly exposed to stimuli challenging their functional integrity. As has already been indicated, the commonest of these are obstruction and contamination with microorganisms. The homœostatic mechanisms may be presented thus: those concerned with the maintenance of patency of the air passages, and those concerned with control of microorganismal contamination.

## The Patency of the Air Passages

Although, in unusual circumstances, foreign material sufficient to obstruct bronchi or bronchioles may enter the bronchial tree, this is rare compared with the presence of mucous secretion produced *in situ*. This viscous secretion normally forms a thin layer on the epithelial surfaces down to the smaller bronchioles, where both mucus production and cilia cease to be found. The normal method of disposal of this secretion (Florey, 1954) is by ciliary action driving the "mucous sheet" towards the trachea, whence it spills over into the pharynx and is swallowed.

This system is frequently overloaded, by either increased mucus production or decreased ciliary activity. In these circumstances mucus accumulates to form local masses. Such a mass in the tactile sensitive area of the trachea and large bronchi elicits the cough reflex, coughing being maintained until the mass is no longer present in this area. Ordinarily this results in expulsion. The efficacy of coughing in clearing this mucus obviously depends on the sensitivity of the reflexogenic zone (depressed by local anæsthetics), the activity of the reflex arc in the central nervous system (depressed directly in sleep, in coma and by drugs such as morphine, or indirectly by pleural pain), and weakness of the respiratory muscles (reduced by wasting or paralysis). Pathological changes causing stenosis in the region of the larynx, trachea or main bronchi or paralysis of the cords will obviously have a similar prejudicial effect on the cough mechanism.

The maintenance of airway patency is not, however, a simple matter of ciliary action and coughing. During bronchography, when cough is suppressed, the opaque medium is seen to flow into the bronchial tree by gravitational force, to enter the small bronchioles with inspiration and to retreat a little with expiration, thus eventually reaching the alveoli (Di Rienzo, 1949). The cilia are powerless to impede its progress. Even hours after, particularly if coughing is weak, many bronchioles are still obstructed. Similarly, masses of mucus not expelled completely by the violent expiration of a cough may be carried peripherally by the subsequent deep inspiration, the result being obstruction of numerous bronchioles (Archibald and Brown, 1927).

In either circumstance collapse of the lung distal to the completely obstructed bronchioles does not ordinarily occur, and subsequent coughing expels the obstructing plugs.

This maintenance of aeration distal to the block is effected by collateral ventilation; without collateral ventilation bronchiolar obstruction would inevitably lead to air absorption and collapse. The basis of collateral ventilation is now well understood. Anatomically, the terminal units of the lung are not end organs, there being minute defects in the alveolar walls known as alveolar pores whereby adjacent lobules are connected. These are generally regarded as being present at birth upon the establishment of respiration, and as increasing with age in both number and size (Macklin, 1936; Loosli, 1937). The effective extent of collateral ventilation is thus a lobe, except where fissures are incomplete,

when it may be two lobes or a lung (van Allen and Adams, 1930; van Allen and Jung, 1931).

Experimental investigation of collateral ventilation has established several points relevant to this presentation.

First, collateral ventilation is extraordinarily efficient. Lindskog and Bradshaw (1934), using dogs, showed that extensive bronchiolar obstruction was not ordinarily accompanied by significant decrease in arterial oxygen saturation. Many subsequent authors have stressed the efficiency of this means of maintaining aeration in bronchiolar obstruction and its clinical significance.

Secondly, in experiments performed on animals (Lindskog and Bradshaw, 1934; Baarsma and Dirken, 1948) and in man (Baarsma, Dirken and Huizinga, 1948), even with quiet respiration the pressure in the distal air passage was higher than in that proximal to the site of obstruction. This pressure gradient rose greatly with coughing.

This observation is explained by "air-trapping" in collaterally ventilated areas due to narrowing or to tortuous exit passages on expiration. The efficacy of this mechanism in expelling bronchiolar plugs needs no emphasis.

Thus, in circumstances in which the ciliary system is overloaded and discrete masses of mucus form in the respiratory tract, clinical, radiological and pathological evidence indicates that bronchiolar obstruction by these masses is a common event, but that such plugs do not ordinarily interfere significantly with lung function and are usually rapidly expelled.

Collapse of alveoli following bronchiolar obstruction therefore should be regarded as an unusual sequel. As van Allen and Jung (1931) were among the first to point out, air absorption and collapse will occur only if collateral ventilation is greatly reduced; it follows automatically if a lobe with complete fissures has its lobar bronchus occluded. Lobular collapse follows bronchiolar obstruction only when there is either gross general reduction in collateral ventilation, such as occurs in rapid shallow breathing, or when the alveolar pores are occluded by fluids, especially inflammatory exudate (van Allen, Lindskog and Richter, 1930, 1931).

## CONTAMINATION BY MICROORGANISMS

The frequent exposure of the bronchial tree to microorganisms compared with the relatively low incidence of established infection indicates the efficacy of the normal homœostatic mechanisms. Because of considerable differences, viral and bacterial agents should be treated separately.

### *Viral Contamination*

The role of homœostatic mechanisms in viral infections of the respiratory tract is difficult to assess in the present state of knowledge. For instance, that exposure to cold alters host function and results in an apparently higher incidence of clinical infection is widely accepted; but the manner in which this occurs is conjectural.

The influence of dose of virus applied to the surface of the respiratory tract epithelium in determining the severity of infection suggests that host mechanisms play some part in determining the nature of the ensuing infection. Whether these mechanisms operate partly by simple physical effects as in bacterial infections is, as yet, impossible to say. Work that has been done on this point suggests that homœostatic mechanisms influence more the spread of the infection than the initial invasion by the virus (Fazekas de St. Groth and Donnelly, 1950 *a* and *b*).

### *Bacterial Contamination*

The commoner bacteria do not ordinarily produce inflammatory lesions, not because these organisms do not frequently enter the air passages—they commonly do—but rather because, along with intermingled mucus, most of them are rapidly expelled. Despite this mechanism for their removal, a proportion of inhaled air-borne bacteria and particles of similar size will always reach the respiratory part of the bronchial tree (Scott *et alii*, 1949). The air of hospital wards contains most of the common bacterial pathogens, so we must conclude that, as bacterial infection does not occur in all who enter the ward, such organisms reaching the respiratory part of a normal lung are usually rapidly destroyed or inhibited.

These organisms will produce lesions only if they proliferate, and proliferation can occur only in an area in which humoral and cellular reactions such as phagocytosis are limited. Accumulations of static material in the bronchial tree provide almost the only suitable areas of this type for the initial proliferation of bacteria in the lung. Thus the mechanisms acting to maintain the patency of air passages also exert a control on bacterial contamination. Though air passages of all sizes may be blocked, obstruction is far commoner in the smaller bronchioles. Moreover, the commonest material obstructing bronchioles is mucus, and mucus is a suitable medium for growth and multiplication of bacteria. Attention is therefore logically focussed on the place of acute bronchiolitis in pulmonary infections.

Few clinicians would doubt that obstruction of air passages plays an obvious role in infections due to organisms of the " non-specific " group such as *Strep. viridans*, non-hæmolytic streptococci, and the majority of types of *Diplococcus pneumoniæ*. However, similar considerations apply to organisms of " higher virulence ", such as group A hæmolytic streptococci. Such organisms can be readily grown on culture from the pharynx in streptococcal pharyngitis, and can therefore be assumed to enter the lower part of the respiratory tract in large numbers in this condition. That pulmonary infection is an unusual complication is evident to all. What has not been appreciated is the inference that the mere presence of " highly virulent " organisms in the bronchial tree is insufficient to produce an inflammatory lesion.

## The Pathological Anatomy of Acute Bronchiolitis

Much that has been presented may be examined critically on a morphological level. The anatomy of acute bronchiolitis can be divided into exudative and repair phases. The exudative or acute phase presented here may be considered in terms of the establishment of the lesion and its spread.

### Establishment of the Lesion

#### *Acute Viral Bronchiolitis*

The penetration by the virus of the superficial ciliated epithelial cells is followed by a cycle of intracellular proliferation, death of these cells and spread to adjacent areas (Sanders, 1954). Fazekas de St. Groth and Donnelley (1950 *a* and *b*) have shown, in experimental influenza, that the extent of this spread bears a relation to the amount of specific antibody in the exudate accompanying the inflammatory changes. In a susceptible animal and in man virus infection spreads readily throughout the bronchial tree, even producing necrosis of the alveolar lining (Muckenfuss, McCordock and Harter, 1932).

Following this destruction of surface epithelium inflammation develops with œdema and vascular congestion of the submucosa, together with a predominantly mononuclear cellular response—lymphocytes, macrophages and plasma cells. Bronchioles are frequently occluded in this early stage by cellular debris and exudate containing mucus. These findings, with particular mention or illustration of bronchioles, have been recorded in the majority of known and presumed viral infections, particularly in human influenza by Scadding (1937), by Straub and Mulder (1948), by Mulder and Verdonk (1949) and by Hers (1955), and in measles by MacCallum (1919).

Secondary bacterial infection supervenes frequently, and if such organisms as *Staph. aureus* and group A hæmolytic streptococci are the secondary invaders, the outcome is often rapidly fatal. Mainly because of this, little is known of the pathological anatomy of uncomplicated viral infection in man. However, Hers (1955), by a careful comparison of primary bacterial infection and secondary infection accompanying influenza in man, has shown that ciliated epithelium initially destroyed when virus is present regenerates from the remaining basal layer (even in the presence of secondary infection). Regeneration may be evident on the fourth or fifth day of the illness and ciliated cells can be recognized as early as the fifteenth day.

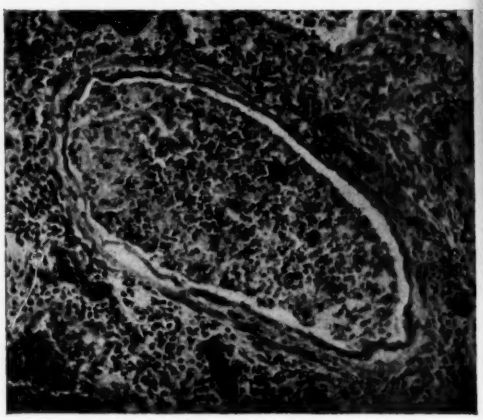

FIGURE I

Fulminating measles pneumonia. The epithelium of this medium-sized bronchiole consists only of a tenuous layer of cells. The lumen contains debris and cellular exudate. (Hæmatoxylin and eosin stain ×90)

In this study no opportunity arose for obtaining early intravenous fixation of the lungs of a patient who had died in the early stages of proven virus infection of the respiratory tract. Most material from such subjects prepared in a routine manner shows extensive artefact epithelial desquamation. However, one example of fulminating measles pneumonia in an infant, was adequate, and showed the classical features of " giant cell pneumonia ". In areas where few bacteria were present, small bronchioles lined by a tenuous layer of epithelium were common (Figure I) ; at other sites, particularly in the region of the terminal

bronchioles, proliferating epithelium was seen at the stage of early stratification (Figure II). No ciliated epithelium was found in any of the larger bronchioles in the block of tissue that was examined.

### *Acute Bacterial Bronchiolitis*

*Primary Bacterial Infection.* Clinical evidence suggests that primary respiratory tract infection by the common pyogenic

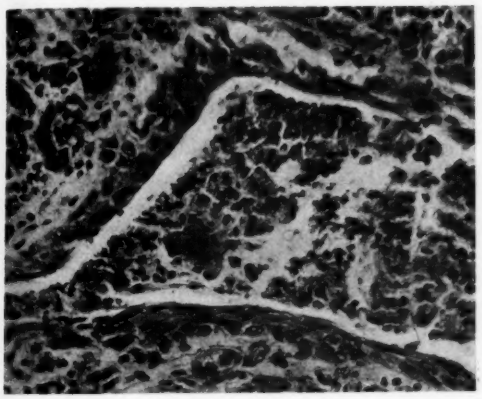

FIGURE II

Fulminating measles pneumonia. A small bronchiole is seen in an area with little secondary infection. The wall in the lower part of the illustration is not cut obliquely, and the proliferating epithelium shows a suggestion of stratification. (Hæmatoxylin and eosin stain. ×240)

bacteria does not occur unless there is stasis of infected material. Further, this stasis arises most commonly by the formation of mucous plugs in bronchioles. Morphological evidence to support this concept is readily accessible. Evidence of the frequency of bronchiolar occlusion by mucus can be gained from the lungs of active patients who die suddenly.

For example, a patient, aged fifty-eight years, having clinically recovered from a cardiac infarct, was ambulant and about to go home when he suddenly collapsed and died within a minute. Intravenous fixation was performed one and a half hours after death, and subsequent macroscopic and histological examination revealed no evidence of acute inflammation in the lungs. However, occasional small bronchioles (about 1%) were occluded by homogeneous mucous plugs (Figure III). Many of these plugs contained scattered Gram-positive cocci and diplococci. It can be concluded that these plugs were of recent formation and, had the patient lived, would have been expelled rapidly.

The characteristics of the mucous plugs can be studied more easily in extreme examples. One such was in a young man who died of asphyxia resulting from a rapidly growing carcinoma in the region of the carina. Both lungs showed extensive patchy occlusion of bronchioles with mucus, but collateral ventilation had kept most of the lung aerated. The mucus extended down into the terminal air passages where, in most cases, it had been ingested by macrophages—a process which can therefore be concluded to occur rapidly. However, there was no evidence of such phagocytosis proximal to the first order of respiratory bronchioles, even in bronchioles in which all the mucus in their more distal branches was intracellular (Figure IV).

Phagocytosis of mucus by macrophages proximal to the respiratory bronchioles is thus a relatively slow process, and, indeed, has been observed only in cases of " dry " bronchiectasis, in which plugs are presumably retained for a long period (Figure V).

It is clear, then, that bronchiolar plugs of mucus occur frequently and ordinarily are rapidly expelled. If circumstances are such that the plug is retained, removal by phagocytosis is unusual. Most plugs contain bacteria,

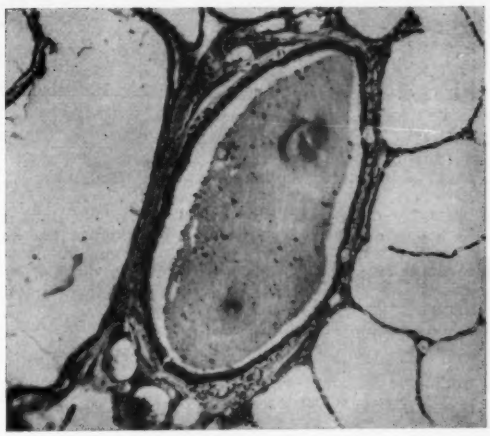

FIGURE III

A medium-sized bronchiole is seen to be occluded by a relatively homogeneous mucus plug containing a few leucocytes. The patient died suddenly with no evidence of respiratory tract infection; few other plugs were found (the dilated structure on the left is the accompanying branch of the pulmonary artery). (Intravenous fixation; hæmatoxylin and eosin stain, ×90)

and the more usual course is the rapid development of a sequence of events in the plug leading to actue bronchiolitis.

This sequence is most easily studied in terminal respiratory infection. In patients who had been bed-ridden and ill for some time before death, all degrees of acute bronchiolitis

were found, depending on the degree and duration of the factors already discussed which interfere with homœostasis in the bronchial tree. The first event is proliferation of bacteria in the plug. Diffusion of the products of bacterial growth and activity elicits an inflammatory response, characterized by both congestion and œdema of the submucosa

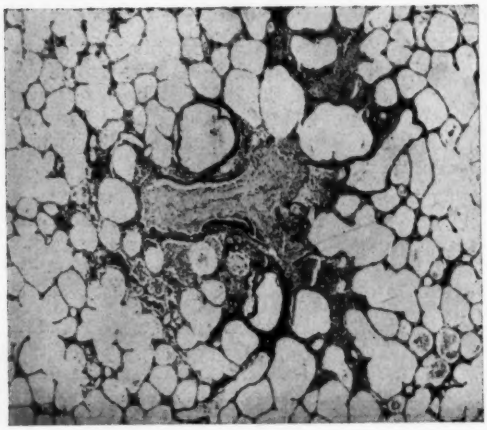

FIGURE IV

Section of two first order bronchioles just distal to the terminal bronchiole, both occluded by infected mucus. Peripherally the mucus has been entirely phagocytosed by macrophages. Aeration has obviously been maintained by collateral ventilation. The patient had a rapidly growing carcinoma occluding the main bronchus of the opposite lung; death was due to extension of growth into the trachea, and asphyxia. (Intravenous fixation; hæmatoxylin and eosin stain, ×24)

and chemotactic infiltration by ploymorphonuclear leucocytes; this is the "mural bronchiolitis" of Engel (1947). As Hers and Mulder (1953) showed, the epithelium remained intact in non-staphylococcal infections, and leucocytes were seen between epithelial cells (Figure VI) and accumulating in the mucous plug (Figure VII).

In many paraffin sections the plug only partially filled the lumen (Figures VI and VII); this is an artefact of paraffin processing, and complete occlusion was obvious in fresh material as well as in frozen or celloidin sections. This artefact has in the past given rise to uninformed speculation as to whether the mucous plug acts as a one-way valve. All evidence indicates that this valvular obstruction is a curious rarity since, when the specimen is examined by other methods, the obstruction is complete in most affected bronchioles.

At this stage occasional bacteria were sometimes seen between the epithelial cells and in the submucosa. Presumably they reach these positions as a result of simple physical factors, and their small numbers suggest that they are rapidly dealt with, as is the case when, for example, similar "non-specific" organisms are liberated into the blood-stream by manipulation of an apical abscess on a tooth. That morphological evidence of invasion and proliferation was not encountered, as would be evidenced by more numerous organisms in the submucosa, emphasizes the relative rarity of established infection by those bacteria which can grow and proliferate in living tissue.

Meanwhile, examination of the plug revealed alterations in the areas where it contained a high density of bacteria and polymorphonuclear leucocytes. The mucus became less homogeneous and, later, gave a negative response to the periodic acid-Schiff reaction. This is regarded as being due to digestion by leucocytic and possibly bacterial proteases.

When the plugs showed indications of numerous damaged leucocytes, irregular masses

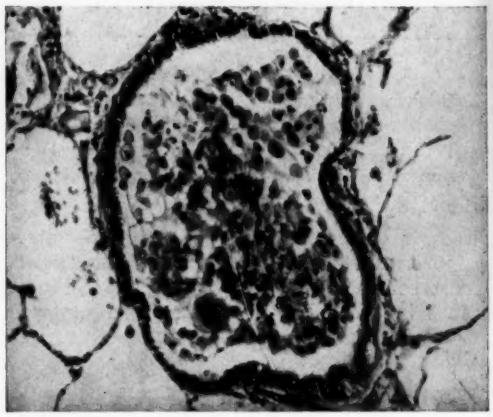

FIGURE V

A small bronchiole from a lobe showing diffuse bronchiectasis. The wall is thin and contained little smooth muscle. The lumen is packed with macrophages containing mucus. Some phagocytic giant cells have formed. (Hæmatoxylin and eosin stain, ×90)

of a material staining deeply with hæmatoxylin were seen (Figure VIII). This material was not stained by the periodic acid-Schiff method, and was stained by the Feulgen method. These staining properties are consistent with the presence of desoxyribonucleic acid, which has been observed to occur in the sputum of patients with bacterial respiratory tract

infections of this type (Armstrong and White, 1950; White, Elmes and Walsh, 1954). Its origin is, therefore, probably in the nuclear remnants of dead leucocytes.

Elsewhere, empty bronchioles showing acute inflammation of their walls were observed. The degree of inflammation was rarely severe, and in these bronchioles the lesions may be interpreted as resolving after expulsion of the infected plug. The observation that the more severe the inflammatory process in a bronchiole, the more frequently a plug was present, also supports this concept of the evolution of bronchiolitis. When collateral ventilation had been abolished by involvement of the surrounding parenchyma the plug was always present, but by this stage the mucus of the plug was usually digested and the lumen of the small bronchiole filled with leucocytes and exudate (Figure IX).

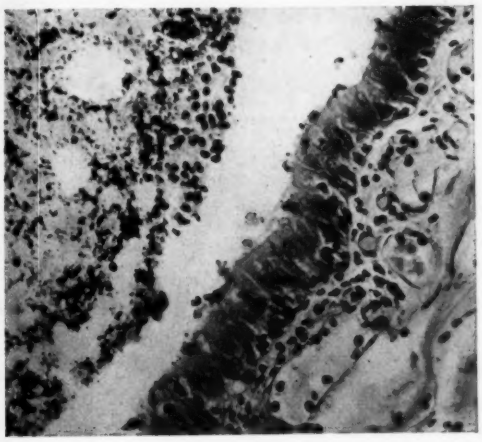

FIGURE VI

Section of bronchiole in which the lumen was completely occluded by the plug which has shrunk during processing. The plug contains huge numbers of bacteria of mixed morphology. The inflammatory reaction is at a very early stage, and polymorphonuclear leucocytes can be seen in the submucosa, between the epithelial cells and on the periphery of the plug. The patient died with terminal acute broncheolitis; non-specific organisms only were grown on culture from the sputum, and no chemotherapy was given. (Intravenous fixation; hæmatoxylin and eosin stain, ×180)

Standard teaching (Coope, 1948; Scadding, 1952) is that bacterial bronchiolitis and bronchopneumonia are "descending infections" from the trachea and main bronchi. However, from clinical observations and the evidence of this investigation, it appears that, apart from some specific conditions—for example, diphtheria—bronchiolitis is the common lesion. Of 30 subjects of terminal infections examined post-mortem, acute bronchiolitis was present in all, but acute tracheobronchitis was seen in only 17.

It is therefore difficult to believe that infection in the air passages usually begins

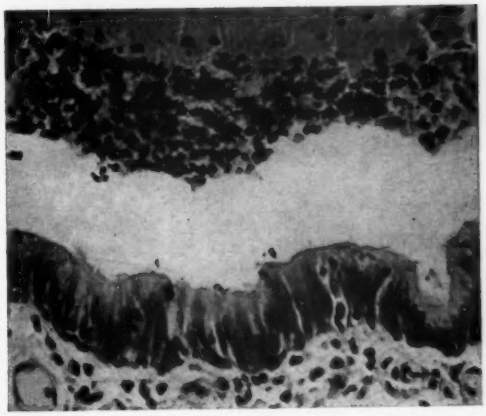

FIGURE VII

The section was taken from the same lobe as that in Figure VI. In this bronchiole there is a more dense accumulation of leucocytes in the plug. In the plug there were many bacteria, many of which were intracellular, but none were found in the bronchiolar wall. (Intravenous fixation; hæmatoxylin and eosin stain, ×360)

proximally and "descends". It seems more likely that the acute tracheobronchitis is, like bronchiolitis, usually due to stasis of infected mucus, and occurs only as a terminal event because only in moribund or comatose patients is a degree of stasis likely to be reached at which the infected mucus lingers even in the larger air passages. It is rather that the level of stasis (and therefore the level of infection) "ascends" than that the infection "descends". The view that the infection in the larger tubes is due to absorption from proliferating bacteria and disintegrating cells in static mucus is also supported by the observation that in none of the foregoing 17 subjects of terminal tracheobronchitis was there histological evidence that bacteria were proliferating in the submucosa.

*Bacterial Bronchiolitis Secondary to Viral Infection.* In the active adult primary bacterial bronchiolitis is rare, whereas infection secondary to viral disease is extremely common and, indeed, can be said to occur in virtually every

case of acute coryza—an indication of how readily the bacterial flora of the naso-pharynx enter the lower part of the respiratory tract. Establishment of infection in such circumstances can be attributed to destruction of the cilia, which abolishes entirely the " sheet " method of disposal of mucus and exudate. Combined with this are an excessive amount of debris

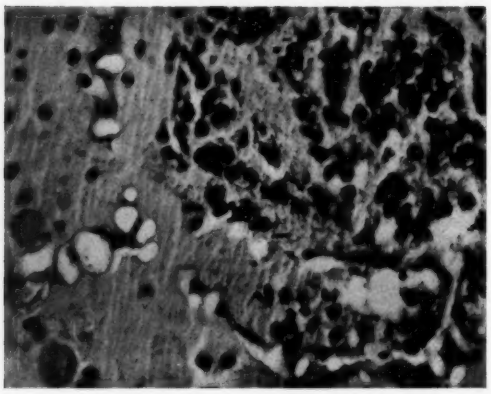

FIGURE VIII

An infected plug is illustrated in the region of a distorted respiratory bronchiole. On the left the mucus is in more distal passages, and two macrophages distended with mucus can be seen. On the right, in the lumen of the respiratory bronchiole, leucocytes are more abundant and many were necrotic. In between the polymorphonuclear leucocytes are dark masses and streaks of the material considered to be desoxyribonucleic acid. The mucus in this area has been largely digested. (Intravenous fixation; hæmatoxylin and eosin stain, ×360)

and exudate in the bronchial tree and reduction of lumina in air passages. These factors result in frequent bronchiolar obstruction and increased difficulty of expulsion of the plugs. Proliferation of bacteria in the plugs may well be aided also by the lack of epithelium, which allows closer contact with the plug with the submucosa—the source of oxygen supply.

In the common type of infection by " non-specific " bacteria, in the active adult, acute bronchiolitis is not apparent clinically except for the presence of mucopurulent sputum and its persistence. In such a person most bronchioles remain clear, but at times a " tightness in the chest " occurs which can be relieved by coughing. Heavily infected plugs expelled from the bronchioles may remain on the surface of the bronchi and trachea, now denuded of cilia, for sufficient time to produce some inflammation in their walls. Clinically this constitutes " acute bronchitis "—a name distracting attention from the more significant bronchiolar disease. This concept, based primarily on deduction, is difficult to examine directly. Patients who die suddenly of other causes at this stage of such an infection are rarely encountered; but reports of the evolution of secondary infection associated with virus diseases in experimental animals afford confirmatory evidence. In work such as that of Shope (1931, 1936) on swine influenza and that of Muckenfuss, McCordock and Harter (1932) on vaccinia infection in rabbits, the polymorphonuclear cell response to secondary bacterial infection was first seen in the bronchioles.

The pathological anatomy of bacterial infection secondary to viral disease of the respiratory tract has been investigated in various combinations, both in man and in animals. Among the viruses influenza has received most attention, and the work of Straub and Mulder (1948), of Mulder and Verdonk (1949), of Hers and Mulder (1951, 1953) and of Hers (1955) is outstanding, in that the evolution of the acute lesions has been

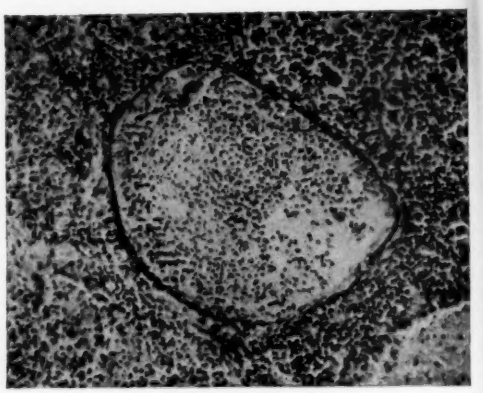

FIGURE IX

Staphylococcal pneumonia; this lesion appeared to be resolving, and no extracellular bacteria were demonstrated in the area. The central bronchiole was recognized only by the surviving sub-epithelial elastic net. No smooth muscle fibres were seen in the wall over the next 500 μ of its length. (Elastin stain, ×90)

studied in carefully prepared material. In summary, non-staphylococcal pyogenic infections have been shown perhaps to delay, but not to prevent, the regeneration of epithelium after virus infection, and the histological features are largely those of acute viral bronchiolitis with a superimposed polymorphonuclear cell response to the bacterial

infection (Hers, 1955). Engel's (1947) "proliferate mural bronchiolitis" is a stage of this process.

Staphylococcal infections, however, show characteristic changes in both primary and secondary infection.

In the material reported here necrosis of areas of epithelium (including the basement membrane) due specifically to this organism was common. Despite the persistence of organisms in the plug, these "ulcerated" areas showed evidence of healing (Figure X).

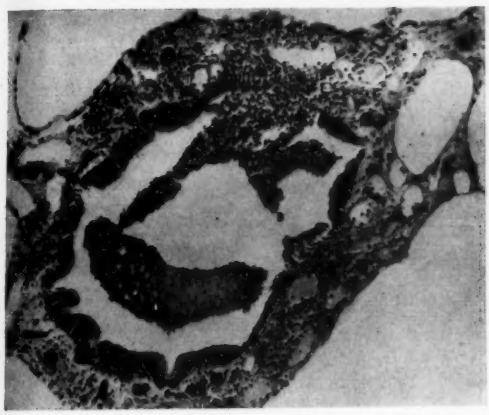

FIGURE X

Staphylococcal bronchiolitis with ulceration. The patient died with a terminal infection, and staphylococci were grown on culture from the sputum. Large Gram-positive cocci were present in the plug (which has shrunk considerably with processing) and in the necrotic area in the upper part of the picture. Here the epithelium is absent, and an elastin stain demonstrated fragmentation of elastic fibres at this site and at the lower right side of the bronchiole where reepithelialization is occurring despite the persistence of the inflammatory process. (Intravenous fixation; hæmatoxylin and eosin stain, ×90)

As Hers (1955) pointed out, staphylococci were never found in the bronchiolar wall if the epithelium was intact; they were found only where there was necrosis.

These observations suggest that staphylococci in the plug invade the bronchiolar wall only where the concentration of toxin, presumably coagulase, has been sufficient to cause necrosis, and then are found only in and on the periphery of these necrotic areas. The action of a specific exotoxin thus differentiates the staphylococcal from the common non-staphylococcal pyogenic bronchiolitis.

This necrosis frequently involved the specialized tissues in the wall. Smooth muscle was rapidly destroyed and the subepithelial elastic tissue net was also damaged (but possibly less rapidly); generally, the destruction of wall structure was permanent. Dissolution of smooth muscle and elastic tissue in staphylococcal infections is in strong contrast with non-staphylococcal pyogenic bronchiolitis, in which in infection of similar duration evidence of this damage was minimal; only in prolonged obstruction with infection was there obvious loss of specialized tissue; then it was often not severe.

These observations emphasize that most bronchiolar infections are not associated with bacterial proliferation in living tissue. That a few pyogenic organisms such as group A hæmolytic streptococci can proliferate in living host tissue is not denied; but the circumstances of establishment of infection are so similar to those associated with "non-specific" infection, that it is reasonable to conclude that the basic process of bronchiolar obstruction with infected material occurs as an essential preliminary to the development of the high local concentration of organisms necessary to the invasive process.

## SPREAD OF THE ACUTE LESION

### *Non-staphylococcal Acute Bacterial Bronchiolitis*

Spread to the larger air passages has been considered already; but the inflammatory process may extend locally to produce what is generally described as bronchopneumonia. This condition is not as common as the clinician would expect. In most cases investigated by the writer and diagnosed clinically as "terminal-bronchopneumonia", usually little more than acute bronchiolitis was found. Indeed, widespread bronchopneumonic consolidation was seen in only five of 30 such cases.

Examination of serial sections suggests that the process begins with the appearance of exudate in the passages distal to obstruction in the terminal bronchioles and in alveoli adjacent to the bronchiolar wall. The lymphatic vessels of the wall were often dilated, and this exudate may be thought of as representing an excess beyond what the lymphatic vessels can remove.

In many cases the exudate filled only part of the lobule, so that major passages remained air-containing, whereas alveoli arising from their walls were fillled with exudate (Figure XII). Subsequent development of this lesion produced

an appearance usually described as " interstitial pneumonia ", particularly when this alveolar exudate was partly absorbed and the cellular infiltration was sufficiently dense to give the impression that the " alveolar walls " were thickened. Elastic and reticulin stains demonstrated that these " alveolar walls " consisted of several layers of collapsed alveoli, the

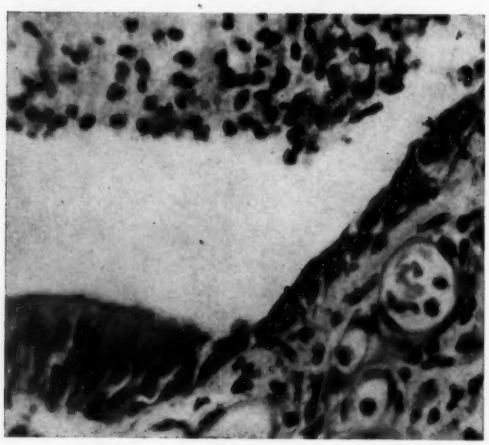

FIGURE XI

A higher power photomicrograph of the lower right part of the wall of the bronchiole shown in Figure X, illustrating regenerating epithelium extending over the wall from the normal area on the left. (Intravenous fixation ; hæmatoxylin and eosin stain, ×360)

interstices being filled with exudate and cells ; the remaining air-containing spaces were mostly pre-alveolar passages.

More rapid exudate formation may fill the whole lobule—this was often apparent in another part of the same tissue section (Figure XII). In the absence of progressive infection, air absorption and collapse often paralleled this development, and lobular collapse was the dominant feature. If on the other hand the infective process spread, the continuing exudation and cellular infitration resulted in consolidation. Such areas of consolidation may become confluent—an event which occurs rapidly in cases in which the organisms are proliferating freely in the newly formed exudate. This type of spread has been observed on many occasions ; Robertson and Uhley (1936) and Loosli (1937) illustrate it well, their photomicrographs showing, in pneumococcal pneumonia, alveolar exudate containing numbers of unphagocytosed bacteria, with relatively few polymorphonuclear leucocytes. As Loosli (1937) shows, spread can occur through the alveolar pores, and, with the involvement of a whole lobe, one form of lobar pneumonia can result—that due to confluence of areas of bronchopneumonia.

The other form of pneumococcal lobar pneumonia typically affects the active adult, and has a dramatic onset usually associated with dyspnœa and considerable apprehension, followed over the ensuing hours by the gradual appearance of toxæmia and fever.

The classical experiments of Blake and Cecil (1920) on monkeys, in which infection was produced by endotracheal injection of one cubic centimetre of dilute bacterial suspension, has been quoted constantly as evidence of a primary bacterial infection in which obstruction plays no part (Scadding, 1952) ; but, as Coryllos and Birnbaum (1929) pointed out, there is much evidence for an obstructive basis. Features such as the onset, so similar to post-operative lobar collapse, and the initial decrease in size of the lobe are strongly suggestive.

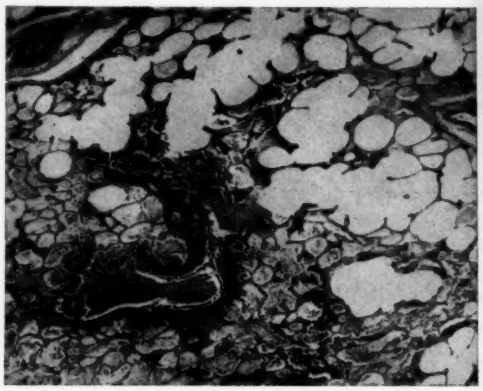

FIGURE XII

Divisions of the bronchiole (on the left) supplied most of the area illustrated. The bronchiole shows typical acute bronchiolitis with a densely cellular occluding plug. More distal passages (lower left) are filled with exudate containing a few cells. Above, several distal passages are patent, their aeration being obviously due to collateral ventilation from adjacent lobules. However, many of their alveoli are filled with exudate giving the appearance, in this area, of early " interstitial " pneumonia. (Hæmatoxylin and eosin stain, ×20)

To explain the observation that the classical condition is restricted to the young active adult, the allergic hypothesis was propounded. Even authors who favour it (Reimann, 1938) admit that there is no evidence to support it. The main objection to Coryllos and Birnbaum's hypothesis, that the condition is due to

obstruction of the lobar bronchus, also is that this does not explain the predilection of the condition for the active adult.

Scadding (1952) has indicated that a virus infection frequently precedes this condition, and if lobar pneumonia is regarded as a rare complication of acute viral bronchiolitis, an hypothesis emerges which satisfies clinical and pathological observations.

During the course of such viral bronchiolitis the patient may by chance be exposed to virulent (phagocytosis-resisting) pneumococci, such as types I and II. The infant, the elderly and the ill may not expel infected bronchiolar plugs rapidly enough, and acute bronchiolitis or even bronchopneumonia may supervene.

The active adult, on the other hand, normally expels these plugs rapidly, and indeed, can deal with considerably more infected mucus in the bronchial tree without significant secondary infection becoming established. However, in one circumstance expulsion fails dramatically. This rare event is almost simultaneous bronchiolar obstruction throughout a whole " unit " of collateral ventilation by inspiration of a considerable mass of infected mucus. This admittedly must be uncommon; but this type of lobar pneumonia is a rare complication of coryza even in patients carrying the appropriate bacteria in the naso-pharynx (Scadding, 1952). In these circumstances, there will be simultaneous establishment of acute bacterial bronchiolitis throughout the " unit "—the lobe. The collapsing lobe rapidly fills with exudate through which bacteria spread.

That this is an unlikely phenomenon in all but the active adult is simply because a highly effective mechanism for expulsion of bronchiolar plugs is a necessary prerequisite, otherwise scattered acute bronchiolitis will supervene at any earlier stage. As this hypothesis of widespread bronchiolar obstruction appears to be in accord with clinical and pathological observations, it is necessary to reexamine Blake and Cecil's animal experiments (1920), interpreted as indicating that obstruction is not essential. First, these authors noted that not all monkeys infected became diseased, even though they were not immune. Secondly, these workers stated that the earliest lesions were in the region of terminal bronchioles, despite their subsequent conclusion that invasion occurred through the walls of the large bronchi—a conclusion for which inadequate evidence was adduced. Thirdly, they always injected one millilitre of fluid into the trachea. Fourthly, as Coryllos and Birnbaum (1929) pointed out, when these experiments were repeated the lesion was considered to be a rapidly confluent bronchopneumonia.

Therefore these experiments should not continue to be quoted as a proven example of non-obstructive pulmonary infection.

### *Staphylococcal Acute Bronchiolitis*

In staphylococcal acute bronchiolitis, the process of spread beyond the bronchiole is initially similar to that in the non-staphylococcal form. It is modified, however, by extensive necrosis of tissues. The commonest form exhibited numerous small abscesses, in the centre of which a necrotic bronchiole could be demonstrated by elastic tissue stains, even at a relatively late stage of development (Figure IX). Thrombosis of both pulmonary and bronchial vessels was frequent in these areas, and more extensive lesions showed thrombosis of the vessels adjacent to the recently formed abscesses. That the basic lesion is acute bronchiolitis is apparent even macroscopically.

## SUMMARY

The establishment of the common air-borne acute pulmonary infections is reviewed, and observations made on the morphology of the early lesions are interpolated. These observations indicate that acute bronchiolitis is the basic lesion in the establishment of bacterial infection. Although most infections of the respiratory tract are initially viral in origin, definite proof of this is rarely obtained in a fatal case either before or after death. Necessarily, this restricts and confuses the significance of observations made on apparently simple bacterial infections; but evidence is adduced which suggests that present concepts of the establishment of air-borne bacterial infection of the lungs are in many ways unsatisfactory.

The establishment of acute bacterial bronchiolitis can be seen to depend not so much on the characters of the bacteria in the air passages as on disturbance of host mechanisms, the most important result of which is retention of infected plugs in the bronchioles.

Although the direct demonstration is difficult owing to the paucity of material, the finding of occasional plugs in the lungs of an apparently well patient who has died suddenly, indicates that scattered bronchiolar obstruction occurs even in health—that is to say, in clinically symptomless individuals. It will occur very much more extensively under the following conditions: (i) if the

" sheet method "of disposal of mucus fails, owing to interference with ciliary activity or its abolition by virus infection or ischæmic necrosis; (ii) if excess fluid material accumulates in the bronchial tree, as results from viral inflammation or aspiration of foreign material.

In normal circumstances the homœostatic mechanisms operative in the air passages will rapidly clear these obstructions; but if these mechanisms are disturbed, then retention of infected plugs and inflammation may follow. The establishment of infection is therefore related to the following: (i) The presence of " virulent " organisms in the obstructing plug. This is regarded as insufficient alone to result in the establishment of infection. (ii) Highly viscous material forming the plug as in asthma or fibrocystic disease of the pancreas. (iii) The size of the bronchiolar lumen. Normally obstruction occurs more readily towards the terminal bronchiole. Infants and children have smaller air tubes. The lumen may be reduced by viral inflammation or spasm. (iv) Interference with collateral ventilation. This may follow obstruction of the lobar bronchus or, when small bronchi or bronchioles are affected, blockage of the alveolar pores or depressed respiration. (v) Interference with the cough mechanism, due to such factors as depression of the cough reflex, weakness of respiratory muscles, proximal obstruction in the main air passages or pleural pain.

In these circumstances bronchiolar obstruction will probably persist and bacterial proliferation in the plugs will cause acute bronchiolitis; in the majority of cases there is no evidence of proliferation of organisms in the bronchiolar wall, and it is clear that this phenomenon is not essential to the development of the lesion.

Both the pattern of subsequent changes in the bronchiole and that of spread to passages proximally and distally depend initially more on the duration of retention of the infected plug than on the nature and properties of the organisms in it, although the bacterial species present may subsequently contribute distinctive features to the lesion. If this occurs, the original mode of origin of the infection tends to be obscured; because of this the initial bronchiolar lesion has frequently been ignored, particularly in pneumococcal lobar pneumonia. A unified approach to the pathogenesis of the common air-borne bacterial infections of the lung is therefore presented, in which bronchiolar obstruction by infected plugs is stressed as the common feature.

## ACKNOWLEDGEMENTS

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# THE POST-COMMISSUROTOMY SYNDROME[1]

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THE post-commissurotomy syndrome is the title that has been given by American writers to a remarkable phenomenon observed after some 10% of operations upon the mitral valve. Whilst the title is inapt, it is also non-committal and so reflects our current inability to prove the ætiology or pathology. The syndrome has also been named "pleuro-pericarditis" (Glover), and the "pericardotomy syndrome" (Campbell). Its disturbing frequency and unpleasant character have not received sufficient recognition because of the general satisfaction of physicians and surgeons engaged in mitral valve surgery. As an example of the syndrome, let us consider the case of Mrs. C.M., aged fifty-two years, who in December, 1955, underwent a successful finger fracture of her mitral valve commissures.

Mrs. C.M., aged fifty-two years, had attacks of rheumatic fever at the ages of eleven and twenty-one years, but had experienced no active rheumatic symptoms since. She suffered cardiac embarrassment in her pregnancies at the ages of thirty and thirty-three years. Prior to operation she had noticed exertional dyspnœa and palpitation on mounting steps or during heavy household tasks. She had never had a frank attack of pulmonary œdema, or of hæmoptysis or chest pain. On presentation, she had auricular fibrillation with signs of dominant mitral stenosis, and minor aortic and mitral incompetence. X-ray examination revealed slight cardiac enlargement with undue increase in the size of the left auricle and hilar congestion (Figure I). Right cardiac catheterization revealed a pulmonary artery pressure of 50 millimetres of mercury, systolic, and 25 millimetres, diastolic, and pulmonary capillary pressure of 22 millimetres of mercury. At operation the mitral orifice was found uncalcified and would not admit the finger tip. The anterior commissure was split completely, and the posterior commissure to a lesser degree, with the production of an aperture equivalent to the diameter of almost two fingers. No great force was required, and the thorax was closed without embarrassment. In the first five days after operation her course was uneventful. The lung remained satisfactorily expanded. On the sixth day after operation she developed a severe stabbing pain in the left side of the chest anteriorly, radiating to the back. Her appearance became anxious, and her breathing shallow and rapid. No friction was audible, but it was apparent radiologically that the heart had increased in size (Figure II). The temperature had risen sharply and become remittent with free perspiration. She denied the presence of any joint pain. Penicillin and streptomycin were without effect. Signs of congestive heart failure soon became apparent, and a mercurial diuretic was administered in addition to digitalis. A little blood-stained fluid drained from the wound. Her condition continued to deteriorate until two weeks after operation, when 40 milligrammes of pregniserone per day ("Metacorten"—Schering) was administered. The temperature fell within forty-eight hours and she made a dramatic recovery. Ten days later all signs of heart failure had disappeared, and her cardiac area was much reduced (Figure III). The cortisone dosage was gradually reduced to 10 milligrammes per day under cover of calcium aspirin, 15 grains every four hours. Two days after her last dose of hormone, she suffered a complete relapse. Coarse râles reappeared at the lung bases, and a further chest X-ray examination indicated patchy consolidation of the middle and lower lobes of the left lung. She slowly recovered as the aspirin was continued, and without the aid of cortisone, and has remained well, and much improved in her exercise tolerance (Figure IV and chart, Figure V).

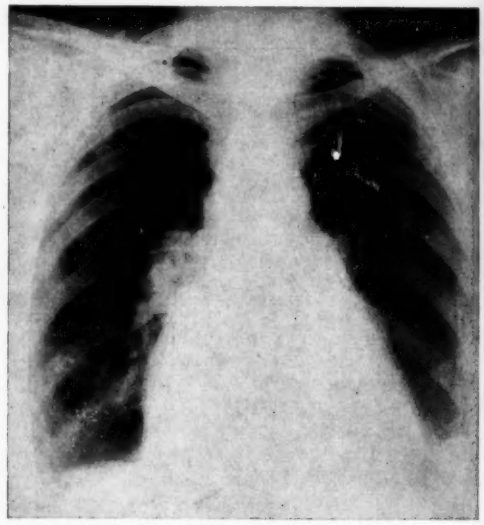

FIGURE I

Teleröntgenogram of patient C.M. before mitral valvotomy

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This case reveals most of the usual features of this syndrome, and shows well the suppressive effect of cortisone. The patient had a typical "pain and fever" syndrome (Wood), which illustrates the usual characteristic sequence of events of these episodes. The sudden onset of sharp, incessant pain in the upper part of the chest, exaggerated by cough and movement, the increasing remittent fever, and the appearance of congestive heart failure, arrhythmia and general misery, are all common features, to which are sometimes added arthralgia, sweating, myalgia and dyspnœa. Physical signs common to all published descriptions and to our own experience are those of pleural effusion and friction, basal râles, tachycardia, relative immobility of the chest, especially on the left side, occasionally visible joint swelling, and the usual external criteria of early congestive heart failure. We have not observed, either in the literature or among our own patients, severe anæmia, copious sputum, rheumatic nodules or rashes. Hæmoptysis, mainly in patients so affected pre-operatively,

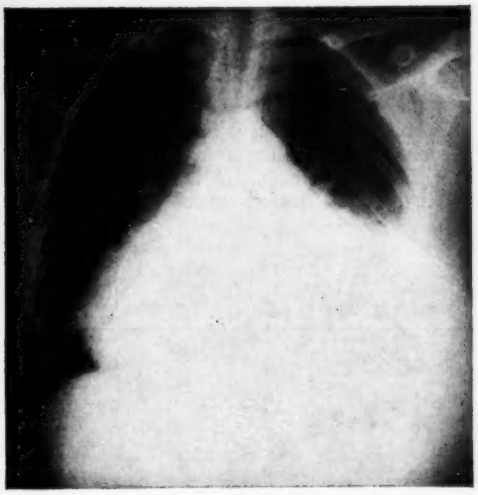

FIGURE II
Teleröntgenogram of patient C.M. at height of post-commissurotomy syndrome

has been described, but we have not encountered it. In their general appearance these patients look weary and uncomfortable, rather than very ill. The chest pain, which is invariable, is severe and unremitting; it is sharp and cutting in nature, and often demands the parenteral administration of powerful analgesics for its relief. It is made worse by movement, and sometimes by deep breathing. Radiographs frequently reveal basal pleural effusions or pneumonitis. Cardiac enlargement is usually apparent.

Our first experiences with this picture occurred early in 1951 among the first cases of mitral valvotomy performed in Sydney, and we were puzzled and worried as to the diagnosis,

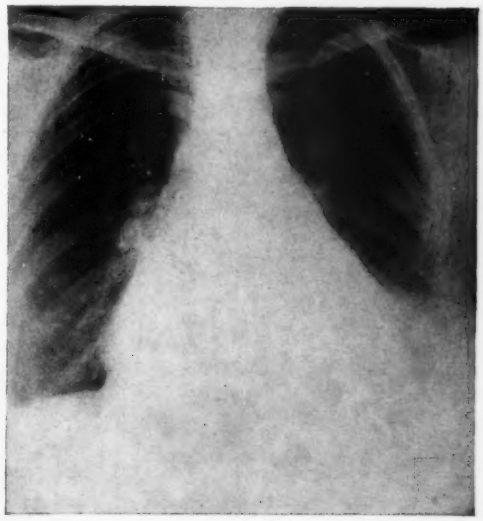

FIGURE III
Teleröntgenogram of patient C.M. during remission of post-commissurotomy syndrome

treatment and results. Impressed by the spiky nature of the temperature chart, we had many blood cultures performed, on varying media and under varying oxygen tensions. All gave negative results, and the full spectrum of antibiotics of those days was completely ineffective. Sometimes an effusion escaped through the partly healed incision, and sometimes we carried out an aspiration, expecting to find signs of degenerated blood clot. At operation the pericardium had been loosely sewn, and any effusion could find its way into the left pleural cavity. When at last all antibiotics were withdrawn as failures, the condition subsided spontaneously. In general, laboratory tests were unhelpful. Leucocytosis was not impressive, the sedimentation rate was only moderately raised, while attempted cultures of the blood and aspirates were invariably sterile. Our total experience of major examples of this syndrome to September, 1955, has been 20 cases in 218 operations (an incidence of 9·1%—Table I). Seventeen of these patients

were females, three males. There were no deaths. Ten patients gave a history of active rheumatism, usually in childhood. Four had had symptoms of acute rheumatism within a year of operation. In only one case was chest pain a feature of such activity. Usually the post-operative fever and chest pain continued for several weeks, but another clinical pattern consisting of short recurrent febrile relapses was sometimes encountered and has been described by other observers. This type may immobilize the patient for periods up to three months.

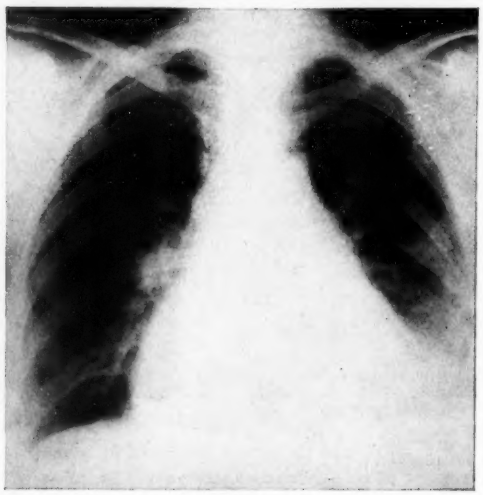

FIGURE IV
Teleröntgenogram of patient C.M. after recovery from post-commissurotomy syndrome.

The incidence of this complication varies from 5% to over 40% (Table II) in the various series described in the literature. The latter figure is certainly too high, and must include a number of minor and incomplete syndromes. These would not usually fall into the accepted definition. The most intriguing aspect of this question is that of ætiology. Pleurisy and pericarditis are too frequent to be minimized, and some observers prefer to designate the syndrome as "pleuro-pericarditis" (Glover). The pain is certainly that of irritation of a serous surface, and small pleural effusions are commonplace, although not necessarily on the side of the thoracic incision. Pleural effusion occurring after the disappearance of that immediately following operation was detected in 10 cases; in two of these the effusion discharged itself through the semi-healed scar. Pericarditis is less clear-cut. Probably many patients after operations involving pericardotomy and handling of the pericardium show electrocardiographic signs of pericarditis. We have not made any controlled observations on this point, but found *S-T* segment elevations and *T* wave changes consistent with acute pericarditis in five of our series. Pericardial friction was heard in four of our 20 cases. The location of the pain in the central chest area, rather than at the lung bases, must be accorded significance. Pericardial effusions have been described, but can occur only in cases in which the syndrome follows after an interval sufficiently long for the pericardial incision to have healed. Dressler has drawn attention to the similarity between the symptoms of idiopathic pericarditis and the post-commissurotomy syndrome. We have not often observed any gross accumulation of pericardial fluid in our patients. It is difficult to state whether signs of early congestive failure are always part of the syndrome since, like post-operative auricular fibrillation, they are also occasionally seen in afebrile patients free from pain. However, we have been impressed by the simultaneous development of congestive heart failure and acute cardiac enlargement in patients with this syndrome (six patients), in whom only slight or moderate cardiac enlargement was evident before operation, and in whom no mitral incompetence was either found at or produced by operation. Joint pains were complained of by 13 patients.

TABLE I
*Post-Commissurotomy Syndrome Incidence*

| Period | Post-Commissurotomy Syndrome | Total Number of Operations |
|---|---|---|
| January, 1952, to September, 1955 | 20 (9·1%) | 218 |
| 1952 | 1 (2·4%) | 41 |
| 1953 | 3 (4·5%) | 67 |
| 1954 | 4 (6·0%) | 66 |
| 1955 (January to September) | 12 (27·0%) | 44 |

TABLE II

| Series | Number of Patients | Post-Commissurotomy Syndrome | Approximate Percentage |
|---|---|---|---|
| Soulie *et alii* | 100 | 10 | 10·0 |
| Glover *et alii* | 41 | 8 | 19·5 |
| Dressler | 60 | 24 | 40·0 |
| Elster *et alii* | 16 | 10 | 60·0 |
| Campbell *et alii* | 45 | 8 | 17·0 |
| Cleland *et alii* | 56 | 7 | 12·0 |
| Verheugt | 37 | 14 | 42·0 |
| Bouvrain | 120 | 10 | 8·0 |
| This series | 218 | 20 | 9·1 |

Râles and poor air entry suggestive of actual lung involvement were recorded in eight cases; but whether this was due to an inflammatory or a congestive cause was difficult to decide (Table III).

Surgical trauma causing a simple healing reaction, resolving hæmothorax, or bacterial infections are not acceptable as the cause of this syndrome, and most observers agree now that these symptoms represent a rheumatic reactivation in the area of operation. This view is based upon the following circumstantial evidence. First, we know that the rheumatic state smoulders on throughout middle age and will occasionally flare up as an attack of polyarthritis or carditis precipitating heart failure or arrhythmia, and that histological evidence of this activity is present in biopsies of the left auricle obtained at mitral valvotomy, in over 50% of the patients. However, the presence of Aschoff bodies in this area bears no relationship to the post-commissurotomy syndrome, but rather is related to preoperative evidence of active rheumatism (Table IV). No finality has yet been reached as to the significance of this finding. Secondly, the syndrome includes signs of pericarditis, of joint pains and swelling, and sometimes of myocardial involvement. The frequent pleural involvement is unlike classical rheumatism, but unlike nodules and rashes, it may be more a feature of the adult form, or it is possible that the pleura may be involved by direct spread from the pericardium. The concept of rheumatic pleurisy and pneumonitis is nowadays rather frowned upon, but cannot be entirely discarded, and in this syndrome we are dealing with a new type of direct mechanical irritation of this area. There is one report of the syndrome under discussion occurring in a case in which pericardotomy alone was per-

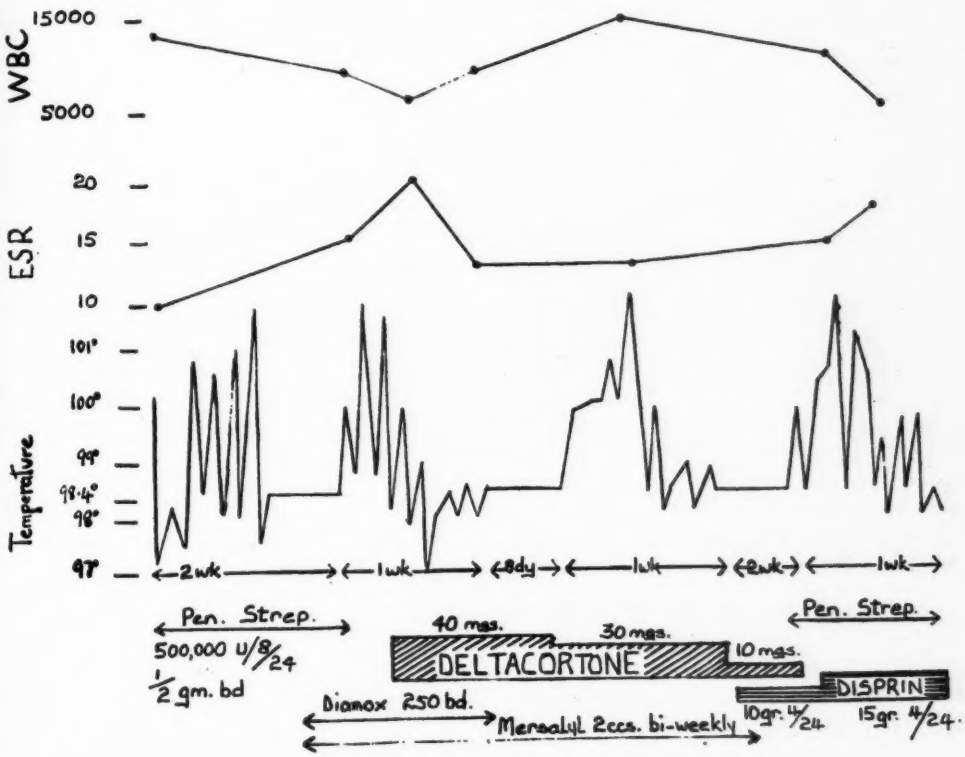


FIGURE V

Course of illness of patient C.M. after mitral commissurotomy. Upper curve, leucocytes; middle curve, erythrocyte sedimentation rate; lower curve, body temperature. Notice the ineffectiveness of antibiotic therapy, and the immediate relapse after slight reduction in dose of "Delta cortisone"

formed. Thirdly, the partial response to salicylates, and the consistent and prompt subsidence following administration of cortisone (Table V), though often temporary, are familiar in acute juvenile rheumatism. Fourthly, the syndrome does not occur with any significant frequency in thoracotomy, cardiotomy and cardiopexy in non-rheumatic patients.

While relapses are common, nearly all observers agree that these events do not interfere with an immediately satisfactory result from valvotomy, and observations to

TABLE III

*Post - Commissurotomy Syndrome: Symptoms and Signs in 20 Cases*

| Symptom or Sign | Number of Cases |
|---|---|
| Fever | 20 |
| Chest pain | 16 |
| Joint pains | 13 |
| Joint swelling | 3 |
| Pericardial effusion | 12 |
| Pericardial friction rub | 7 |
| Electrocardiographic evidence of pericarditis | 5 |
| Pleural effusion | 10 |
| Pneumonitis | 8 |
| Cerebro-spinal fluid findings in immediate post - operative period | 6 |
| Recurrences | 6 (in 5 patients) |

date in our own series give this attitude full support. Few deaths have been reported, and eventual recovery can be confidently anticipated. So far I have not seen any account of autopsy findings. However, it is still exceedingly difficult to assess the status of rheumatic activity in any one patient, and impossible to

TABLE IV

*Incidence of Aschoff Bodies in Auricular Biopsy, September, 1954, to September, 1955*

| Condition | Anschoff Bodies | | | Total |
|---|---|---|---|---|
| | Present | Absent | No Report | |
| Post-commissurotomy syndrome | 6 | 8 | 0 | 14 |
| Others | 23 | 16 | 5 | 44 |

foresee what the future holds in this regard. It appears (Elster *et alii*, 1954) that the only laboratory test of value in diagnosis and management of this syndrome is the titre of antibodies present in the blood to C-reactive protein, a test of which so far we have had no experience. The erythrocyte sedimentation rate and the antistreptolysin O value do not parallel the active phase of this syndrome closely enough to be of real clinical value (Table VI). The all-important questions as to whether the post-commissurotomy syndrome includes acute endocarditis of a degree which accentuates

TABLE V

*Post-Commissurotomy Syndrome: Response to Drugs in 20 Cases*

| Drugs | Response | No Response |
|---|---|---|
| Salicylates | 6 | 4 |
| Antibiotics | ? 1 | 19 |
| Cortisone | 1 | 0 |

resealing of the valve, or whether constrictive pericarditis ever follows, may receive a partial answer as the years pass. In any case it is a most uncomfortable experience for the patient, and trials are now in progress to determine whether its not inconsiderable frequency can be lowered by the prophylactic administration of salicylate or cortisone, and whether its occurrence can be foretold by the presence of C-reactive protein in the blood before operation.

TABLE VI

*Post-Commissurotomy Syndrome: Laboratory Findings in 20 Cases*

| Laboratory Investigation | Results |
|---|---|
| Estimation of erythrocyte sedimentation rate | Normal, 1 case<br>Abnormal, 12 cases |
| White cell count | Normal, 5 cases<br>Abnormal, 8 cases |
| Attempted culture of microorganisms from blood | Unsuccessful, 4 cases<br>Successful, 0 case |
| Attempted pre-operative isolation of group A hæmolytic streptococcus | Unsuccessful, 0 case<br>Successful, 1 case |

Pre-operative investigation of patients by existing clinical and laboratory criteria for evidence of active rheumatism does not, in our experience, ensure a normal post-operative course.

## SUMMARY AND CONCLUSIONS

Attention is drawn to a syndrome consisting of chest pain, fever and joint pains, with evidence of pleural and pericardial irritation which followed 20 operations on the mitral valve among 218 operations at the Royal Prince Alfred Hospital, Sydney. These episodes may follow immediately or some weeks after operation, and indirect evidence suggests that

they represent reactivation of the rheumatic state, provoked by operation. They do not appear to follow other types of operation on sufferers from rheumatic heart disease, and frequently occur many years after the last experience of acute rheumatism. Our own figures are too small for statistical analysis, but a review of the literature suggests no relationship between the syndrome and (*a*) the age or sex of the patient, (*b*) histological evidences of rheumatic activity in biopsy of the auricular appendix, (*c*) the difficulty of the valvotomy, (*d*) the pre-operative history of acute rheumatism, or (*e*) the bacteriology of the pharynx. The syndrome is almost never fatal, and so far does not seem to influence adversely the satisfactory outcome of mitral valvotomy. Symptoms and fever can be abated immediately by the use of cortisone, but relapse can occur on cessation of its administration. No satisfactory objective test exists at present to assist in forecasting this complication, but the test for C-reactive protein is promising and deserves further investigation. Post-operative penicillin prophylaxis, as advocated by American workers, has not been practised in our cases.

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# TREATMENT OF SEVERE ARTERIAL HYPERTENSION[1]:

## RESULTS FROM LONG-TERM USE OF METHONIUM COMPOUNDS WITH AND WITHOUT THE ADDITION OF RESERPINE

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AN evaluation of any treatment for arterial hypertension must take into account the natural history of the condition, particularly in respect to the production of symptoms and duration of life. Recent studies (Master *et alii*, 1952; Hamilton *et alii*, 1954) have shown that the systolic and diastolic blood pressure in a community are continuous variables, and people cannot be sharply divided into "hypertensives" and "normotensives". The possession of a blood pressure above the mean for the population is not always pathological or an indication for treatment. Consequently a statement that by the use of a particular treatment the blood pressure has been "controlled" for a certain time does not necessarily mean that the treatment has been beneficial. However, pronounced hypertension often leads to deterioration in function of certain organs, with resulting symptoms and possible shortening of life.

The difficulties in assessing the value of any therapy in arterial hypertension are readily apparent. Many of the symptoms are poorly defined, and some, such as headache (Stewart, 1953), are held to be largely psychogenic. Usually the prognosis is reasonably good (Palmer *et alii*, 1948; Perera, 1948; Burgess, 1948; Hammarstrom and Bechgaard, 1950), and a lengthy investigation would be required to demonstrate that a remedy prolonged life. These difficulties may be overcome if any therapeutic trial is confined to patients with severe symptoms, and if the investigation includes a large proportion of patients whose expectation of life without treatment is short. This is so in hypertension in the "malignant" phase, characterized by the presence of papilloedema, in which there is an expected mortality after five years of approximately 100% (Keith *et alii*, 1939; Frant and Groen, 1950).

[1] Received on April 30, 1956.

[2] Associate Director, Clinical Research Unit, Alfred Hospital.

The causes of arterial hypertension are probably numerous—both genetic and environmental—and the mechanism by which they act is still imperfectly understood. However, there is evidence that the sympathetic nervous system plays some part in this mechanism. Thus sympathetic nervous blockade produces a greater fall in blood pressure (both in absolute values and proportionally) in hypertensive than in normotensive people (Barnett and Fraser, 1954). In the past, great benefit was claimed to result from lumbo-dorsal sympathectomy (Smithwick, 1948). When Paton and Zaimis (1948) demonstrated that sympathetic ganglion blockade could be obtained by use of the methonium compounds, the hope was raised that similar benefit could be obtained by medical means.

Previous studies of these drugs in the treatment of arterial hypertension have given mainly favourable results (Campbell and Robertson, 1950; Saville, 1950; Smirk and Alstad, 1951; Campbell *et alii*, 1952; Freis, Finnerty *et alii*, 1952; Palmer, 1952; McQueen and Trewen, 1952; Morrison, 1953; Smirk, 1954; Harrington and Rosenhain, 1954; Freis, Partenope *et alii*, 1954), although those obtained by Turner (1950) and Blainey (1952) were unfavourable, and the findings of Sieber and his co-workers (1953) were not encouraging. Other studies have dealt with the use of the methonium compounds in combination with the following other drugs: methonium and hydralazine (Schroeder 1951, 1952, 1954; Schroeder *et alii*, 1953, 1954; Moyer *et alii*, 1953); methonium and rauwolfia compounds (Ford *et alii*, 1953; Smirk *et alii*, 1954; Dennis *et alii*, 1955; Hughes *et alii*, 1955; Doyle *et alii*, 1955; Bain *et alii*, 1955; Perry and Schroeder, 1955); or methonium, hydralazine and rauwolfia (Markovitz *et alii*, 1955). Generally it has been claimed that combined treatment is more beneficial than treatment with methonium compounds alone.

Some of the previous studies have been based on relatively short experience, and in others there is no indication that the patients have been examined frequently by the same observer. In spite of the numerous papers by other authors, it is considered worthwhile therefore to report the following series of cases based on close personal observation of the patients over a reasonably long time.

## MATERIAL AND METHODS

### *Selection of Patients*

It was decided to limit the trial to patients with severe diastolic hypertension, either in the malignant phase, or producing severe hypertensive symptoms such as incapacitating headache or left ventricular failure, and to exclude those with milder hypertension, and those with indefinite symptoms such as mild headache and giddiness. In general we have adhered to these criteria, but have included a few patients who do not fulfil our original rather strict demands. These have been either young persons with severe diastolic hypertension in whom it was considered advisable to forestall the development of the severe complications, or people who had been treated by us by some other method for a long time without relief of their symptoms. Although moderate impairment of renal function has not precluded treatment, we have rejected patients who were in uræmia when first examined.

### *Material*

Over the past five years 80 patients with severe arterial hypertension have received treatment with ganglion-blocking drugs at this unit. Of these, seven have been treated for less than six months, and the records of six have been inadequate for study owing to scarcity of blood pressure reports or inability to obtain periodic assessment (three of these patients are known to be dead, and the fate of the other three is unknown). Of the remaining 67, on whom this report is based, follow-up studies have been made until the time of their death, or for at least six months.

Data on the age and sex of the patients, the diastolic blood pressure level, the phase of the hypertension and the ætiological basis both for the initial 80 patients and for the 67 with satisfactory follow-up investigations are given in Table I.

The severity of hypertensive disease in the cerebral, ocular, cardiac and renal fields was graded 0 to 3. In the case of cerebral, cardiac and renal function, these grades are as follows: 0, no impairment of function; 1, slight impairment (for example, slight abnormality in tests, minimal symptoms); 2, moderate impairment (for example, moderate abnormality in tests and symptoms, or more pronounced abnormality in tests with minimal symptoms); 3, severe impairment.

In the case of the eye, the degree of impairment was made from the grades of hypertensive retinopathy as described by Keith, Wagener, and Barker (1939) grade I and II retinopathy being regarded as grade 1 impairment, grade III retinopathy as grade 2, and grade IV retinopathy as grade 3.

The initial severity of the hypertensive disease in the 67 patients is shown in Table II.

### *Method*

All patients were admitted to hospital for preliminary assessment and adjustment of dosage. Unless there was some definite medical contraindication, they were encouraged to remain ambulant. In addition to routine clinical examination, special investigation was made of the state of their ocular fundi and of their cardiac and renal function. The ocular fundi were examined by an ophthalmologist, and in many instances a pictorial record was made by an artist. The heart was examined by radiography, fluoroscopy and electrocardiography. Renal function was assessed by microscopic examination of the urine, blood urea examination, and urea clearance and concentration tests. Excretion pyelography was performed in most cases. In many instances special tests (intravenous injection of "Regitine" and estimation of pressor amines in the urine) were conducted to exclude phæochromocytoma. Blood pressures were recorded at frequent intervals and charted, and on at least one day an hourly record was made. (The diastolic blood pressure was taken at the point of disappearance of sound.)

After the investigation period, the response to a methonium drug was tested. In the early part of the trial most patients received an intramuscular injection of two milligrammes of hexamethonium bromide ("Vegolysen", M. and B.) per kilogram of body weight. This was considered to produce fairly complete sympathetic blockade, and we compared the effect of this procedure in hypertensive and normotensive subjects (Barnett and Fraser, 1954). More recently we have used pentolinium ("Ansolysen", M. and B.) and have commenced with a relatively small dose (five milligrammes) given subcutaneously.

The type of methonium compound used for continued treatment has varied during the

TABLE I
*Basic Clinical Data*

TABLE Ia
*Age and Sex of Patients*

| Age (Years) | Initial 80 Patients | | | 67 Patients of Follow-up Study | | |
|---|---|---|---|---|---|---|
| | Males | Females | Total | Males | Females | Total |
| 20+ .. .. .. .. | 1 | 3 | 4 | 1 | 2 | 3 |
| 30+ .. .. .. .. | 9 | 7 | 16 | 9 | 3 | 12 |
| 40+ .. .. .. .. | 13 | 15 | 28 | 12 | 13 | 25 |
| 50+ .. .. .. .. | 15 | 13 | 28 | 11 | 12 | 23 |
| 60+ .. .. .. .. | 2 | 2 | 4 | 2 | 2 | 4 |
| Total .. .. | 40 | 40 | 80 | 35 | 32 | 67 |

TABLE Ib
*Initial Diastolic Blood Pressure and Phase of Hypertension*

| Diastolic Blood Pressure (Millimetres of Mercury) | Initial 80 Patients | | | | 67 Patients of Follow-up Study | | | |
|---|---|---|---|---|---|---|---|---|
| | Benign | Malignant | Unknown | Total | Benign | Malignant | Unknown | Total |
| 100+ .. .. .. | 1 | — | — | 1 | 1 | — | — | 1 |
| 110+ .. .. .. | 4 | — | — | 4 | 3 | — | — | 3 |
| 120+ .. .. .. | 7 | 1 | — | 8 | 4 | 1 | — | 5 |
| 130+ .. .. .. | 11 | 4 | 1[1] | 16 | 8 | 4 | 1[1] | 13 |
| 140+ .. .. .. | 13 | 12 | — | 25 | 10 | 12 | — | 22 |
| 150+ .. .. .. | 4 | 11 | — | 15 | 4 | 9 | — | 13 |
| 160+ .. .. .. | 3 | 3 | — | 6 | 3 | 3 | — | 6 |
| 170+ .. .. .. | 2 | 3 | — | 5 | 1 | 3 | — | 4 |
| Total .. .. | 45 | 34 | 1 | 80 | 34 | 32 | 1 | 67 |

[1] Ocular fundi not able to be examined because of medial opacities.

TABLE Ic
*Ætiological Basis of the Hypertension*

| Probable Ætiological Basis | Initial 80 Patients | 67 Patients of Follow-up Study |
|---|---|---|
| Essential hypertension .. .. | 72 | 60 |
| Due to toxæmia of pregnancy .. | 4 | 4 |
| Due to nephritis and pyelonephritis .. .. .. .. | 4 | 3 |
| Total. | 80 | 67 |

trial according to availability. In the early stages only the preparations for oral use were available in adequate quantities. At first we used pentamethonium bromide ("Lytensium Syrup", M. and B) and later tablets of hexamethonium bromide ("Vegolysen", M. and B.) or hexamethonium bitartrate ("Vegolysen T", M. and B.). The drug was administered three times a day before meals. An average single oral dose was one gramme of the pentamethonium bromide (or an equivalent amount of the bitartrate), tablets being crushed and taken in water.

Later, we used injections of hexamethonium bromide with delaying agents ("Vegolysen Retard", M. and B.). Patients gave their own injections at strictly eight-hour intervals. There was a wide variation in the requirements of different patients, and single doses ranged between 15 and 250 milligrammes, the average dose being about 100 milligrammes.

More recently we have used injections of the delayed absorption form of pentolinium ("Ansolysen Retard", M. and B.) and pentolinium for oral use ("Ansolysen" tablets, M. and B.) Again the drug has been given at eight-hour intervals. The dose required is much less than with the hexamethonium compounds; with "Ansolysen Retard" the dose for a single injection has ranged between 2·5 and 50 milligrammes, with an average of about 15 milligrammes, and a single oral dose of "Ansolysen" has ranged between 10 and 400 milligrammes, with an average of about 100 milligrammes. A certain degree of tolerance has occurred with all the drugs, so that the dose has had to be increased, particularly in the first fortnight. An attempt has been made to stabilize the patient on a particular drug during

the period in hospital, and further adjustments have been made as required at the weekly attendance at the clinic.

In the first three years of the trial, the methonium compounds were, for practical purposes, the only anti-hypertensive drugs used. Hydralazine ("Apresoline", Ciba) was used for a short time in a few cases, but the supply was so limited that its use would not influence the course of the condition. More recently we have used reserpine ("Serpasil", Ciba) as an additional form of treatment in some patients, and in a few have replaced the methonium with reserpine, the dose of which has varied from 0·125 to 0·75 milligramme given orally three times a day.

TABLE II
*Severity of Clinical Hypertensive Disease in 67 Patients with Adequate Follow-up Study*

| Region | Severity Grading (see Text) | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | Ungraded |
| Cerebral .. | 37 | 17 | 12 | 1 | — |
| Ocular .. .. | — | 15 | 19 | 32 | 1[1] |
| Cardiac .. | 5 | 31 | 21 | 10 | — |
| Renal .. .. | 26 | 20 | 11 | 9 | 1[2] |

[1] Ocular fundi not able to be examined because of medial opacities.
[2] Not adequately assessed.

In general we have used the most efficacious methonium compound available at the time; however, in order to assess the value of the different compounds, changes have usually been made only after a period of six months on a particular treatment. The order in which treatments have been used in the past has been dictated largely by the order in which they became available: pentamethonium and hexamethonium for oral use, injectable hexamethonium, injectable pentolinium and pentolinium for oral use. Recently, patients have usually been treated primarily with pentolinium given by injection, this being considered the most efficacious remedy. Should this be inadequate or produce severe side effects, the oral administration of reserpine has been added. Should treatment with pentolinium by injection control the hypertension, a change to pentolinium given by mouth with or without reserpine has been tried.

Patients receive the methonium compound, either orally or by injection, at eight-hour intervals (usually at 6 a.m., 2 p.m. and 10 p.m.). Once a week they attend a clinic, held between 8.30 a.m. and 10.30 a.m., and report on their symptoms over the previous week, have their blood pressures recorded in lying, sitting and standing positions, and have adjustments made, if necessary, in the dose of the particular drug used. It is appreciated that the patient's blood pressures fluctuate considerably during the day. In those receiving injections the lowest pressure occurs about one hour after an injection and the highest immediately before the next injection. Patients are examined at the clinic two and a half to four and a half hours after their morning injection, when readings are intermediate in value. The blood pressures, symptoms and treatment are recorded.

Every six months each patient is subjected to a clinical examination, and tests are performed to assess the state of his eyes, heart and kidneys. The findings are taken in conjunction with his blood pressure control and symptomatic state to assess his progress, and a decision is made on his treatment for the subsequent six months.

## RESULTS

### *Follow-up Status*

Of the 67 patients forming the basis of this report (followed until the time of death or for at least six months), 21 have died, 42 are still under supervision (all except one on anti-hypertensive treatment), and four have been lost from supervision. The duration of the follow-up investigation is shown in Table III. Of the four patients lost from supervision, one was a man of poor mentality who refused to cooperate in his treatment, and the other three lived at country centres. Two had poor blood pressure control, and information has

TABLE III
*Follow-up Status of 67 Patients*

| Status | Follow-up Period | | | | | | |
|---|---|---|---|---|---|---|---|
| | Less than Six Months | Six Months | One Year | Two Years | Three Years | Four Years | Five Years |
| Dead .. .. .. | 10 | 11 | 8 | 1 | — | — | — |
| Still under supervision .. | — | 42 | 39 | 31 | 17 | 9 | 1 |
| Lost from supervision .. | — | 4 | 3 | — | | | |

been obtained that one of these had been admitted to a country hospital with features of hypertensive encephalopathy not responding to methonium treatment. The fourth was a young man with severe hypertension in the "malignant" phase. When he was last examined twelve months after starting treatment he was symptomatically well, his blood pressure was well controlled, and his papillœdema had disappeared.

### *Effect on Clinical Picture*

*Deaths and Survivals.*—All except one of the 21 deaths occurred within two years of commencement of treatment. The patients were all severely hypertensive, and with two exceptions the diastolic blood pressure was above 130 millimetres of mercury; 14 were in the "malignant" phase, and seven had severe (grade 3) renal impairment. Causes of death were as follows: a cerebral vascular accident, nine cases; "sudden" death, two cases; renal failure, six cases; cardiac failure, one case; coronary occlusion, one case; dissecting aneurysm, one case; not recorded, one case. The blood pressure control was poor in 18 and fair in three cases. Some patients had been treated solely or mainly with methonium compounds given by mouth—that is, with less effective drugs than those available at present. There was no evidence that the treatment had caused any of the deaths. In spite of the frequently poor blood pressure control, definite symptomatic relief was noted in 16 of the 21 patients.

Forty-two patients at present alive have been followed for various periods from six months to five years. Most of these have returned to, and persisted with, their former occupations. Four patients followed for more than six months have since been lost sight of.

*Symptomatic Relief.*—The effect of treatment on symptoms is shown in Table IV. Here the results of treatment in the patients who have died and in those who are living are tabulated separately. In the living patients the follow-up period has varied from six months to five years, and in some cases the symptomatic relief has varied over this period. For simplicity, the symptomatic effects in the preliminary phase of treatment only are considered. It is recognized that this may be unfair to the treatment, because the more potent drugs used recently may be expected to relieve symptoms more readily than the drugs used earlier. On the other hand, it may be argued that the relief of symptoms with the recent treatment may be due to the passage of time. To discover whether relief has been maintained, presence or absence of symptomatic

TABLE IV
*Effect of Treatment on Symptoms*

| Symptom | Dead Patients. | | Living Patients. | | Total. | | Patients Followed for More than Two Years | |
|---|---|---|---|---|---|---|---|---|
| | Number Affected | Number with Symptoms Reduced or Abolished | Number Affected | Number with Symptoms Reduced or Abolished | Number Affected | Number with Symptoms Reduced or Abolished | Number Affected | Number with Symptoms Reduced or Abolished |
| Headache | 15 | 11 | 33 | 31 | 48 | 42 | 21 | 18 (15)[1] |
| Impaired vision | 15 | 3 | 25 | 15 | 40 | 18 | 16 | 9 (8) |
| Dyspnœa on exertion | 9 | 4 | 18 | 17 | 27 | 21 | 14 | 9 (8) |
| Paroxysmal dyspnœa | 5 | 5 | 9 | 9 | 14 | 14 | 9 | 7 (7) |
| *Angina pectoris* | 2 | 2 | 4 | 3 | 6 | 5 | 2 | 2 (2) |
| Blackouts or dizziness and other minor cerebral disturbances | 3 | 1 | 8 | 8 | 11 | 9 | 4 | 4 (4) |
| Loss of energy | 6 | 2 | 15 | 8 | 21 | 10 | 10 | 3 (3) |
| Nervous tension | — | — | 13 | 5 | 13 | 5 | 12 | 7 (6) |

[1] The numbers in parentheses in the final column refer to the patients in whom the symptom was abolished.

relief has been noted at the time of their last review in those patients followed for more than two years. The striking and lasting relief of headache, dyspnœa on exertion, paroxysmal dyspnœa and minor cerebral disturbances is noteworthy. There was striking improvement in vision in those patients with papilloedema. However, the proportion of the total with improved vision is low, owing to the fact that in some of the patients who died survival was sometimes too short for improvement to occur, and some patients had lesions such as retinal vascular accidents from which no improvement could be expected.

*Progress of the Hypertensive Disease.*—In the 21 fatal cases, death usually occurred within twelve months of the start of treatment and was usually due to a cerebral vascular accident.

Progress of the grade of clinical hypertensive disease in the 46 survivors is shown in Table V and Figure I. It is apparent that there is a decrease in proportion of patients with severe grades (2 and 3) of disturbance of cerebral and ocular systems. This is noted particularly in the eyes, in which grade 3 disturbance (papilloedema), which is common initially, has almost disappeared after one year. Figure II shows the dramatic improvement which may occur in the ocular fundus. The cardiac condition tends to improve up to the first year, after which no further improvement occurs, although on the other hand there is not much tendency to deterioration. The reduction in heart size which may sometimes occur during treatment is illustrated in Figures III and IV. The proportion of patients with the various grades of renal impairment remains fairly constant. It thus appears that in those patients who survive the first twelve months or so, the tendency of organ function is either to improve (cerebral, ocular and in the early period, cardiac) or remain stationary (renal).

Most patients experienced some lowering of blood pressure, which was more pronounced when standing than when lying, with an intermediate value when sitting. With pentamethonium or hexamethonium given by mouth, the reduction in blood pressure was often temporary, and after several weeks or months the pressures returned towards their original values, in spite of an increased dose of the drugs. With injection of hexamethonium or of pentolinium, and often with pentolinium given by mouth, it was usually possible to achieve a persistent therapeutic effect. The postural fall in blood pressure, although responsible for a reduction in the average blood pressure and therefore regarded as an essential part of the therapeutic effect (Smirk, 1950, 1953), is also a nuisance to the patient, because it causes faintness when he is performing his

TABLE V

*Progress of Hypertensive Disease in 46 Surviving Patients with Adequate Follow-up Study*

| System | Grade of Impairment | Number of Patients | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Initial | At Six Months | At One Year | At Two Years | At Three Years | At Four Years | At Five Years |
| Cerebral .. .. | 0 | 26 | 34 | 35 | 28 | 17 | 7 | 1 |
| | 1 | 11 | 8 | 3 | 1 | — | — | — |
| | 2 | 8 | 2 | 1 | — | — | — | — |
| | 3 | 1 | — | — | — | — | — | — |
| | Not assessed | — | 2 | 3 | 2 | — | 2 | — |
| | Total .. | 46 | 46 | 42 | 31 | 17 | 9 | 1 |
| Ocular .. .. .. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | — |
| | 1 | 12 | 21 | 21 | 18 | 12 | 4 | — |
| | 2 | 16 | 17 | 15 | 11 | 3 | 3 | 1 |
| | 3 | 18 | 5 | 2 | — | 1 | — | — |
| | Not assessed | — | 3 | 4 | 2 | 1 | 2 | — |
| | Total .. | 46 | 46 | 42 | 31 | 17 | 9 | 1 |
| Cardiac .. .. | 0 | 5 | 4 | 10 | 7 | 3 | 1 | — |
| | 1 | 21 | 28 | 18 | 10 | 6 | 3 | — |
| | 2 | 14 | 11 | 10 | 11 | 6 | 2 | — |
| | 3 | 6 | 1 | 1 | 1 | 2 | 1 | 1 |
| | Not assessed | — | 2 | 3 | 2 | — | 2 | — |
| | Total .. | 46 | 46 | 42 | 31 | 17 | 9 | 1 |
| Renal .. .. .. | 0 | 22 | 15 | 11 | 13 | 6 | 2 | 1 |
| | 1 | 16 | 25 | 15 | 9 | 8 | 4 | — |
| | 2 | 6 | 4 | 9 | 5 | 2 | 1 | — |
| | 3 | 2 | 2 | 2 | 2 | 1 | — | — |
| | Not assessed | — | — | 5 | 2 | — | 2 | — |
| | Total .. | 46 | 46 | 42 | 31 | 17 | 9 | 1 |

daily tasks. In assessing the blood pressure control we have considered blood pressure with the patient recumbent, and therefore the results will not appear as favourable as those of other authors, who have paid more attention to the blood pressure on standing. The blood pressure control was considered "good" if the diastolic pressure was consistently below 110 millimetres of mercury, "fair" if it was mainly between 110 and 120 millimetres; and "poor" if it was mainly above 120 millimetres. The blood pressure control in blocks

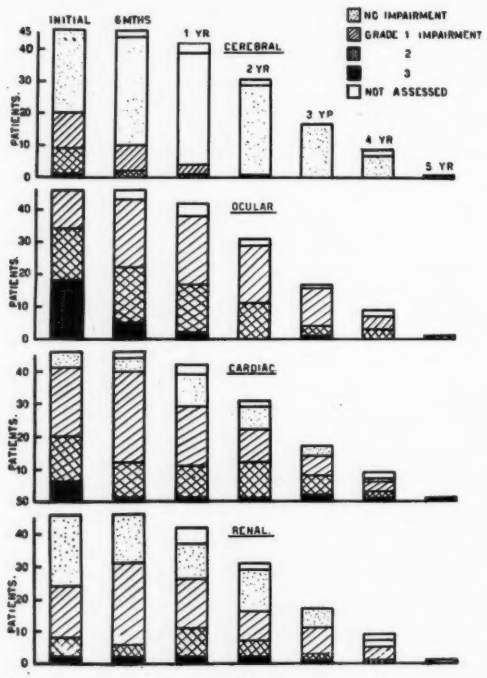


FIGURE I

Progress of hypertensive disease in cerebral, ocular, cardiac and renal fields in 46 surviving patients

of six months' treatment of patients with the various drugs is shown in Table VI and Figure V. (A few blocks in which recordings were infrequent or treatment was not consistent have been omitted.) For the same block, the symptomatic state was assessed as "good", "fair" or "poor", and the side effects as "slight", "moderate" or "severe".

The results shown in Table VI give an indication only of the effectiveness of the various drugs, because they were used on some patients in different phases of the treatment, the usual order in the early (and surviving) members of the clinic being methonium given orally, injection of methonium, injection of pentolinium, pentolinium given orally, combination of one of the two last-mentioned with reserpine. Later members usually commenced on the available drug considered most effective at the time. It is noteworthy that with methonium given by mouth, blood pressure was usually "poor", with the other treatments the proportion of "good" and "fair" rises, but it is only with pentolinium given by mouth *plus* reserpine that "good" or "fair" blood pressure control is achieved in practically all cases. In spite of the high proportion of "poor" blood pressure control with certain treatments, the symptomatic state was usually judged "good" or "fair" with all treatments. Side effects were usually classified as "slight" or "moderate" with all treatments. The highest incidence of "severe" side effects occurred with treatment with methonium by injection or pentolinium by injection. With treatment with pentolinium given by mouth *plus* reserpine, in spite of the favourable effects on blood pressure and symptoms, severe side effects are almost non-existent. In fact, Table VI indicates that this is a very satisfactory form of treatment. In the patients treated with reserpine alone, control of blood pressure and symptomatic relief had previously been easily maintained with pentolinium given orally *plus* reserpine. It appeared that in these patients the hypertension had reached a mild phase, and it is therefore not justifiable to use the figures in Table VI to compare the results from reserpine alone with those from the other drugs or combinations of these with reserpine.

*Side Effects and Complications.*—Some side effects from the use of ganglion-blocking drugs occurred in practically all patients. These included postural faintness, dry mouth, difficulty of accommodation for near vision, "glare" in strong light and muscular weakness. They were controlled to some extent by symptomatic measures: prescription of the parasympatheticomimetic drugs pilocarpine or carbachol, use of appropriate spectacles, aperients. When reserpine was added to the treatment, side effects sometimes occurred from this, particularly lethargy and a gain in weight. However, the dose of the ganglion-blocking agent was usually reduced, with pronounced lessening of its particular side effects. Reference has already been made to the almost complete absence of severe side effects with pentolinium given orally *plus* reserpine, in spite of a good therapeutic response.

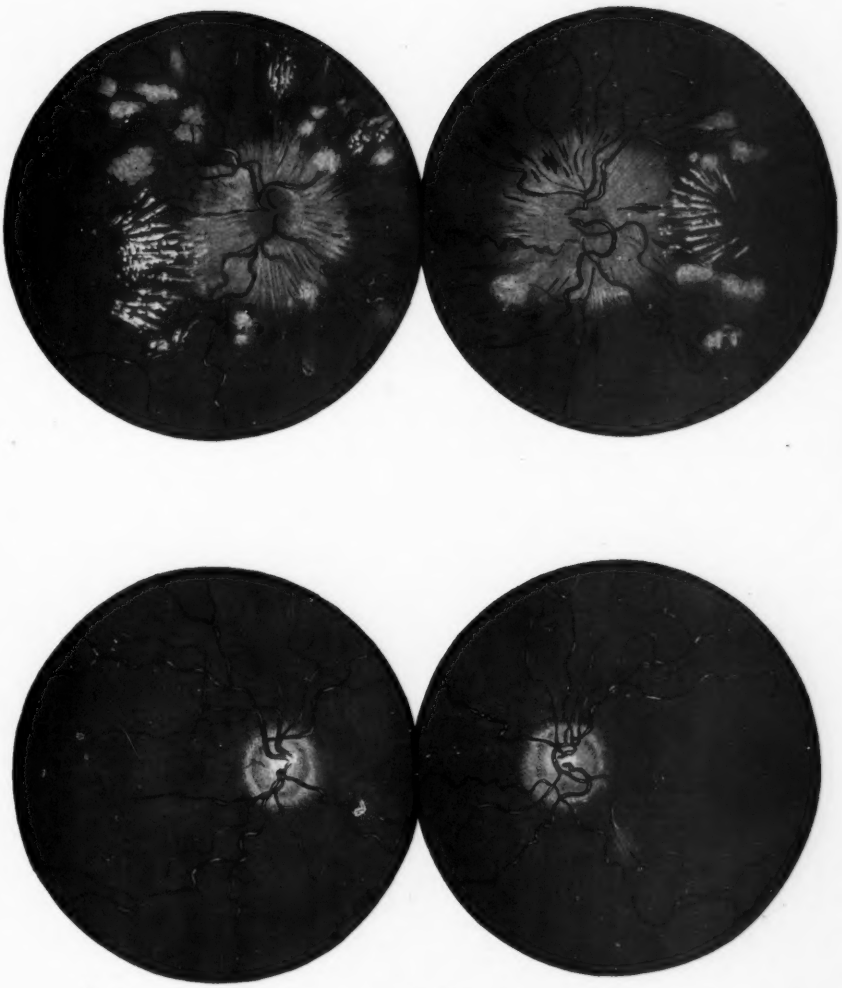

FIGURE II

Artist's painting of ocular fundi of a patient with hypertension in the " malignant " phase before treatment (upper picture) and after approximately twelve months' treatment with methonium compounds (lower picture). Man, aged thirty-nine years ; severe headache and nervous tension and pronounced impairment of vision. Blood pressure before treatment, 260 millimetres of mercury, systolic, and 146 millimetres, diastolic

Early in the use of the methonium compounds it was feared that pronounced reduction in blood pressure might be deleterious, since an adequate blood flow through structurally

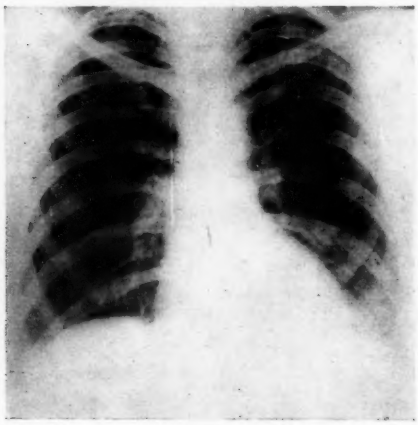

FIGURE IIIA
X-ray pictures of heart of patient H.F. before treatment

narrowed vessels requires a high pressure. Harm would most likely occur in important organs which were also subject to pronounced degenerative changes, particularly the brain,

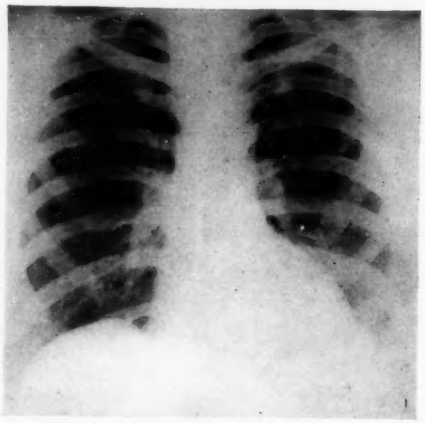

FIGURE IIIB
X-ray pictures of heart of patient H.F. after seven months' treatment with ganglion-blocking drugs

eyes, heart and kidneys. It was feared that low blood flow might produce not only deterioration in function, but also a tendency to thrombosis.

Nine patients have died from a cerebral vascular accident. In only four of these cases was a necropsy obtained, and in each this revealed a cerebral hæmorrhage. In eight of the nine patients the blood pressure control prior to the vascular accident was regarded as "poor" and in one "fair". This indicates that a fatal vascular accident is more likely with a high than with a low blood pressure. Two patients at present alive have suffered from major vascular accidents while under treatment.

A man, aged forty-four years, had suffered from a cerebral thrombosis producing right-sided hemiparesis four weeks before commencing treatment with injections of pentolinium. Blood pressure control was only fair. Three months after commencing treatment

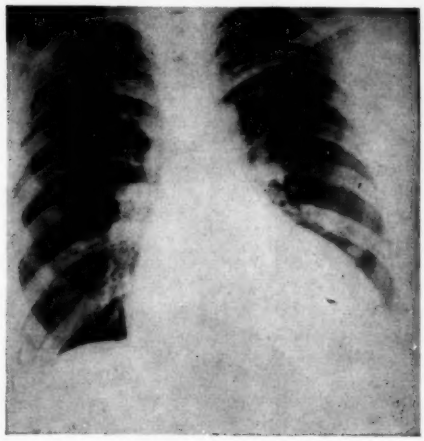

FIGURE IIIC
X-ray picture of heart of patient H.F. after approximately five years' treatment

he had another attack of right-sided hemiparesis. This began in the early morning before he had given himself his injection.

A man, aged forty-seven years, had presented with hypertension in the "malignant" phase, and had been under treatment with ganglion-blocking agents for fifteen months, the most recent treatment being with injections of pentolinium and reserpine given by mouth, which produced good blood pressure control. He then suffered from a hæmatemesis and melæna requiring his admission to hospital and treatment with blood transfusion. Three days after his admission, he noted in the morning that his left arm was paralysed. His blood pressure was then 175 millimetres of mercury, systolic, and 115 millimetres, diastolic. He later developed facial paresis and paresis of his right leg.

There is no evidence from our series that the use of ganglion-blocking agents produces an increased incidence of cerebral vascular accidents. In fact, with the better blood pressure control achieved with the modern drugs, this accident has become less common.

Retinal vascular accidents have also been infrequent in patients under treatment. Two patients have suffered from large vitreous hæmorrhages producing blindness in one eye. One patient has suffered from an occlusion of his central retinal artery.

A man, aged fifty years, with severe hypertension had commenced treatment with pentolinium some two months after a vascular accident in his right eye (diagnosed as a thrombosis of his right upper temporal vein). Eighteen months after commencing treatment he noted an attack of loss of vision in his right eye lasting for two hours and another attack on the following day, the loss of vision on this occasion being permanent. The arteries of the right ocular fundus

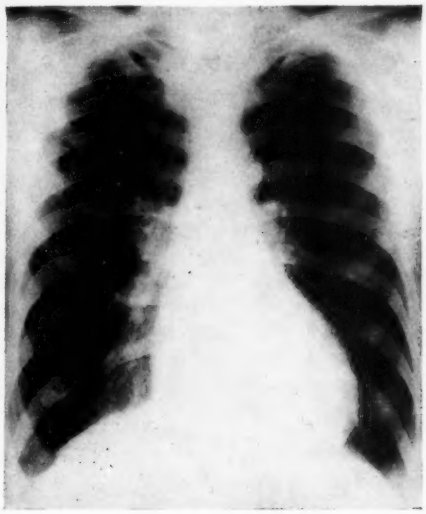

FIGURE IVA
X-ray picture of heart of patient R.L. before treatment

were narrower than on the left, and there was also a large hæmorrhage to the temporal side of the disc. Thrombosis of the central retinal artery was diagnosed. Papaverine was injected into the right internal carotid artery, and tolazoline (" Priscol ", Ciba) was injected behind the right eyeball. These measures produced no benefit, and the patient was left with practically complete loss of vision in his right eye. Prior to this episode the patient's blood pressure control had been poor (the blood pressure during recumbency when he was examined at the clinic being usually about 220 millimetres of mercury, systolic, and 120 millimetres, diastolic). On each occasion the sudden loss of vision occurred half an hour after an injection of pentolinium. It is possible that the retinal arterial occlusion was precipitated by the sudden fall in blood pressure following injections.

Only one patient has suffered (and died) from a cardiac infarction when under treatment. Two other patients died " suddenly ", but there are not enough clinical details for diagnosis. One patient experienced anginal

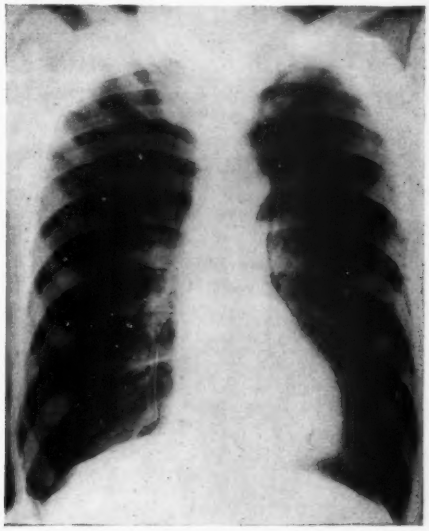

FIGURE IVB
X-ray picture of heart of patient R.L. after six months' treatment with ganglion-blocking drugs

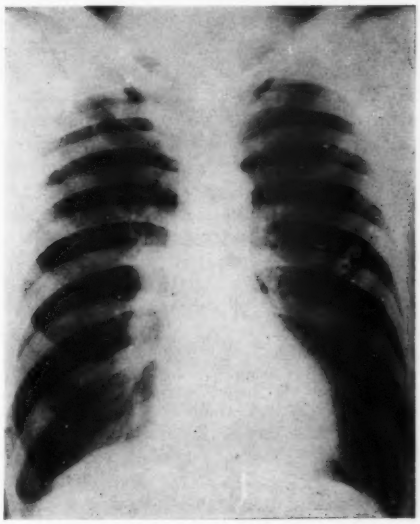

FIGURE IVC
X-ray picture of heart of patient R.L. after approximately three years' treatment

pain when the blood pressure was low after injections of hexamethonium, while on the

TABLE VI

*Blood Pressure Control, Symptomatic Relief and Side Effects*

| Treatment | | Treatment Blocks[1] | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Methonium by Mouth | Injection of Methonium | Pentolinium by Mouth | Injection of Pentolinium | Pentolinium by Mouth *plus* Reserpine by Mouth | Injection of Pentolinium *plus* Reserpine by Mouth | Reserpine by Mouth |
| Blood pressure control | Good | 2 | 6 | 9 | 7 | 14 | 4 | 8 |
| | Fair | 12 | 24 | 10 | 12 | 20 | 8 | 2 |
| | Poor | 37 | 27 | 11 | 13 | 2 | 3 | 0 |
| | Total | 51 | 57 | 30 | 32 | 36 | 15 | 10 |
| Symptomatic state | Good | 21 | 31 | 21 | 26 | 24 | 9 | 3 |
| | Fair | 23 | 19 | 6 | 4 | 9 | 5 | 7 |
| | Poor | 7 | 7 | 3 | 2 | 3 | 1 | 0 |
| | Total | 51 | 57 | 30 | 32 | 36 | 15 | 10 |
| Side effects | Slight | 30 | 26 | 16 | 8 | 20 | 7 | 5 |
| | Moderate | 20 | 24 | 12 | 18 | 15 | 7 | 5 |
| | Severe | 1 | 7 | 2 | 6 | 1 | 1 | 0 |
| | Total | 51 | 57 | 30 | 32 | 36 | 15 | 10 |

[1] One treatment block = treatment of one patient for six months.

other hand several patients with this symptom were relieved. There is no indication from this series that treatment with ganglion-blocking agents increases the tendency to cardiac infarction or *angina pectoris*.

Before commencing this study, we had noted that when a ganglion-blocking agent was given to a hypertensive patient with pronounced renal impairment, a fall in blood pressure was associated with a pronounced diminution in urine output leading to death in uræmia. We have therefore used the drugs cautiously in the treatment of patients with pronounced renal impairment, giving a smaller dose than usual and aiming at only a moderate reduction of blood pressure. Diminished urine output indicates that the dose is too high. Six of our patients have died in uræmia. All of these had hypertension in the malignant phase (as indicated by papillœdema), and in four the renal function was grossly impaired (grade 3) before treatment was begun. There is no indication that the treatment contributed to their deaths.

In several cases, records of fluid balance and daily weights indicated fluid retention, sometimes to the extent of seven or eight litres, and associated peripheral œdema (Fraser and Lowe, 1954). In some instances this required treatment by the conventional methods of bed rest, restriction of salt intake and exhibition of diuretics, but was not regarded as a contraindication to the continued use of the ganglion-blocking compounds, although the dose was sometimes reduced temporarily.

There have been various Intercurrent illnesses, apparently not related to the treatment, and probably not more frequent than in the general population. These included gastro-intestinal hæmorrhage in two cases, in one treated by gastrectomy.

Other workers have reported, among patients treated with ganglion-blocking drugs, instances of paralytic ileus (Bourne and Hosford, 1951), prolonged hypotension (Finnegan and Trounce, 1952) and severe hypotension and paralytic ileus or copious vomiting (Mackey and Shaw, 1951; Hirson and Kelsall, 1951), sometimes with fatal results. Our patients have fortunately so far escaped these complications. However, three have suffered from intestinal obstruction, in one treated by decompression of the bowel through a Miller-Abbott tube, and in two by abdominal operation and freeing of adhesions. All the patients had been previously subjected to an abdominal operation. The obstruction was probably caused by a loop of the bowel becoming dilated by the depressant action of the drug on its musculature, and then becoming kinked over an adhesion. Ganglion-blocking agents should be used with caution in the treatment of patients who have had abdominal operations. If their use is imperative, aperients or parasympatheticomimetic drugs should be given regularly to prevent overloading the bowel.

We have not encountered any definite lung changes as described by Doniach and his co-workers (1954). One patient developed scattered opacities throughout the upper part

of each lung while receiving treatment; these have since cleared.

Recently, attention has been drawn to the high incidence of dissection of the aorta in patients with severe hypertension treated with ganglion-blocking agents (Beaven and Murphy, 1956). Two of our patients have experienced an aortic dissection (in one case, fatal) while under treatment. However, it is not possible to state whether these incidents were complications of the treatment or of the disease.

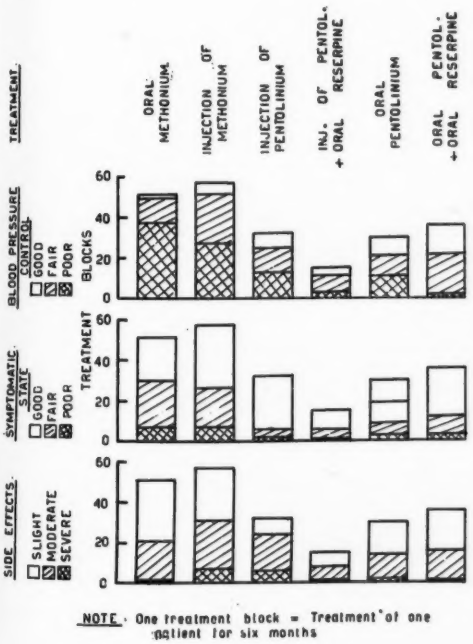


FIGURE V

Blood pressure control, symptomatic state and side effects in " treatment blocks " (of one patient for six months) with ganglion-blocking drugs without and with addition of reserpine

### *Results in " Malignant " Hypertension*

Since it is only in hypertension in the " malignant " phase, with its known serious prognosis, that one can judge the effect of treatment on the course of the disease from a study extending over only a few years, the results from the use of ganglion-blocking drugs in this condition deserve special mention. Thirty-two patients were in the " malignant " phase of hypertension (as judged by the presence of papillœdema) at the commencement of treatment.

Brief data concerning these patients are given in Table VII, and the period of survival is shown graphically in Figure VI. All were severely hypertensive, usually with a diastolic blood pressure above 140 millimetres of mercury. In addition to the pronounced retinal changes, including papillœdema, there were usually signs of hypertensive disease of the heart and kidneys and sometimes of the brain. Fourteen of these patients have died, 16 are living and still under supervision, and two have been lost sight of, each after treatment for over one year. Of the series, 23 of 32 (70%) have survived for more than one year, and 12 (40%) for more than two years.[1]

Without treatment, only 20% would be expected to survive for one year and 10% for two years (Keith *et alii*, 1939). Table VII shows the present condition of the 16 survivors. In most both the symptomatic state and the blood pressure control are good. Most are receiving oral treatment with pentolinium or pentolinium *plus* reserpine. With the exception of three patients (one of whom has been treated for less than one year), papillœdema has disappeared. Hæmorrhages and exudates have become much less numerous. There has been little change in the cerebral or renal status. When any change has occurred in the cardiac condition, this has been in the direction of improvement. Most of the patients are carrying on their normal activities. It would seem that in most cases the progress of the disease has been altered, and that it has been converted from a " malignant " to a " benign " phase.

## DISCUSSION

To crystallize our opinions as a result of treatment with ganglion-blocking drugs and the indications for their use, we may attempt to answer three questions.

### *Is the Treatment Effective?*

Our experience indicates that ganglion-blocking agents are effective in lowering blood pressure in most cases of severe hypertension. When methonium is given orally, tolerance to the action of the drug occurs and in most cases control of blood pressure is soon lost. With the injectable forms of methonium and with pentolinium and, in many instances, with pentolinium given by mouth, prolonged blood pressure control can be achieved. Better blood pressure control is obtained when

[1] Some patients who have not passed the one-year or two-year survival period are still living; so the final survival figures will be better than these.

TABLE VII

*Brief Data on 32 Patients with Hypertension in the "Malignant" Phase Treated with Ganglion-Blocking Agents*

| Patient | Age (Years) | Sex | Ætiology[1] | Blood Pressure on Admission to Clinic[2] | System Impairment | | | | Survival Period | Mode of Death | Present Condition of Living Patients | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Cerebral | Ocular | Cardiac | Renal | | | System Impairment | | | | Clinical State | Blood Pressure Control | Treatment |
| | | | | | | | | | | | Cerebral | Ocular | Cardiac | Renal | | | |
| D.McD. | 43 | M. | E. | 234/142 | 1 | 3 | 1 | 1 | 1 year 2 months | Renal | — | — | — | — | — | — | —— |
| C.D. | 40 | F. | E. | 260/146 | 1 | 3 | 2 | 2 | 1 year 11 months | Cardiac | — | — | — | — | — | — | —— |
| V.K. .. | 38 | M. | E. | 220/146 | 0 | 3 | 1 | 0 | 4 years 10 months | — | 0 | 1 | 1 | 0 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| H.F. .. | 42 | F. | E. | 240/150 | 0 | 3 | 3 | 0 | 4 years 9 months | — | 0 | 2 | 1 | 1 | Good | Good | Pentolinium by mouth |
| E.P. .. | 54 | F. | E. | 260/180 | 0 | 3 | 3 | 3 | 4 years 8 months | — | 0 | 1 | 2 | 2 | Good | Fair | Pentolinium by mouth *plus* reserpine by mouth |
| M.H. | 40 | F. | E. | 256/140 | 0 | 3 | 1 | 1 | 2 months | Cerebral | — | — | — | — | — | — | —— |
| V.L. .. | 50 | F. | E. | 260/165 | 2 | 3 | 1 | 3 | 2 months | Renal | — | — | — | — | — | — | —— |
| E.C. .. | 50 | M. | E. | 230/130 | 0 | 3 | 2 | 2 | 4 years 2 months | — | 0 | 1 | 1 | 2 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| O.S. .. | 51 | F. | E. | 260/150 | 2 | 3 | 2 | 1 | 1 year 7 months | Cerebral | — | — | — | — | — | — | —— |
| D.D. .. | 27 | F. | N. | 250/145 | 0 | 3 | 1 | 3 | 1 year 1 month | Renal | — | — | — | — | — | — | —— |
| F.M. | 50 | F. | E. | 212/150 | 2 | 3 | 2 | 1 | 4 years 1 month | — | 0 | 1 | 2 | 1 | Fair | Fair | Pentolinium by mouth *plus* reserpine by mouth |
| C.S. .. | 39 | M. | P.N. | 230/132 | 0 | 3 | 1 | 2 | 1 year 11 months | Renal | — | — | — | — | — | — | —— |
| M.P. .. | 59 | F. | E. | 245/135 | 0 | 3 | 3 | 3 | 2 years | ? | — | — | — | — | — | — | —— |
| J.H. .. | 53 | M. | E. | 250/130 | 0 | 3 | 1 | 2 | 4 months | Cerebral | — | — | — | — | — | — | —— |
| E.M. .. | 43 | F. | E. | 260/178 | 1 | 3 | 2 | 2 | 5 months | Cerebral | — | — | — | — | — | — | —— |
| G.S. .. | 37 | M. | E. | 260/170 | 0 | 3 | 1 | 1 | 3 years 6 months | — | 0 | 1 | 1 | 0 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| L.A. .. | 39 | M. | E. | 260/146 | 0 | 3 | 1 | 0 | 3 years 1 month | — | 0 | 1 | 0 | 1 | Fair | Fair | Injections of pentolinium *plus* reserpine by mouth |
| B.H. .. | 50 | F. | E. | 286/160 | 1 | 3 | 3 | 2 | 1 year 3 months[3] | — | — | — | — | — | — | — | —— |
| R.L. .. | 46 | M. | E. | 228/140 | 0 | 3 | 2 | 1 | 3 years 1 month | — | 0 | 3 | 1 | 1 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| T.Br. .. | 58 | M. | E. | 235/125 | 0 | 3 | 2 | 3 | 1 month | Cerebral | — | — | — | — | — | — | —— |
| P.D. .. | 58 | M. | E. | 230/140 | 0 | 3 | 2 | 2 | 11 months | Cerebral | — | — | — | — | — | — | —— |
| J.C. .. | 63 | M. | E. | 240/150 | 1 | 3 | 1 | 3 | 2 months | "Sudden" | — | — | — | — | — | — | —— |
| J.B. .. | 37 | M. | E. | 275/158 | 1 | 3 | 2 | 0 | 2 years 6 months | — | 0 | 2 | 1 | 0 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| M.H. .. | 52 | F. | E. | 260/140 | 2 | 3 | 2 | 1 | 2 years 6 months | — | 0 | 2 | 0 | 1 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| R.B. .. | 59 | F. | E. | 280/154 | 1 | 3 | 2 | 0 | 2 years 5 months | — | 1 | 2 | 2 | 1 | Good | Good | Pentolinium ny mouth *plus* reserpine by mouth |
| A.McD. | 47 | M. | E. | 255/145 | 0 | 3 | 1 | 0 | 2 years | — | 0 | 2 | 0 | 1 | Poor[4] | Fair | Pentolinium by mouth *plus* reserpine by mouth |
| H.G. .. | 59 | M. | E. | 240/150 | 0 | 3 | 2 | 1 | 1 year 6 months | — | 0 | 2 | 1 | 2 | Fair | Good | Injection of pentolinium *plus* reserpine by mouth |
| F.F. .. | 46 | M. | E. | 240/140 | 0 | 3 | 1 | 2 | 1 year 7 months[3] | — | 0 | 2 | 1 | 2 | Poor[5] | Poor | Injection of pentolinium *plus* reserpine by mouth |
| T.Ba. | 29 | M. | E. | 185/140 | 0 | 3 | 1 | 0 | 1 year 3 months | — | — | — | — | — | — | — | —— |
| F.L. | 48 | M. | E. | 260/160 | 0 | 3 | 3 | 3 | 7 months | Renal | — | — | — | — | — | — | —— |
| A.E. .. | 38 | M. | E. | 230/155 | 0 | 3 | 1 | 0 | 1 year 1 month | — | 0 | 3 | 1 | 0 | Good | Good | Pentolinium by mouth *plus* reserpine by mouth |
| M.W. | 50 | M. | E. | 230/130 | 1 | 3 | 1 | 1 | 11 months | — | 0 | 3 | 1 | 1 | Fair | Fair | Pentolinium by mouth *plus* reserpine by mouth |

[1] E. = essential; N. = nephritic; P.N. = pyelonephritic.
[2] Systolic/diastolic; millimetres of mercury.
[3] These two patients were lost from supervision after the times shown.
[4] Is under treatment for tuberculosis of the spine.
[5] Marked disability following cerebral thrombosis.

reserpine is used in combination with pentolinium.

Dramatic relief of symptoms usually occurs, particularly of headaches, impaired vision and dyspnœa. This is achieved at the cost of some side effects, but these are usually less troublesome than the symptoms they have replaced.

The treatment has not prevented a certain number of deaths from hypertensive disease. In the survivors, the evidence of hypertensive disease has either decreased (in cerebral and ocular spheres) or shown little change (in the cardiac or renal spheres). The most pronounced benefit has been noted in patients with hypertension in the "malignant" phase, among whom there has resulted increase in survival, pronounced symptomatic relief, and, in the survivors, improvement in the patients' general condition. These results are essentially in accord with those recently reported by Smirk (1954).

*Which Patients Should be Treated?*

The availability of a potent agent for the reduction of blood pressure should not mean that all patients with blood pressures above a certain arbitrary normal level should be treated. As has been pointed out earlier, recent work shows that blood pressure, like many other attributes, is a continuous variable, and that there is no sharp line between the normal and the abnormal.

It is therefore important to consider not only the height of the blood pressure, but also the

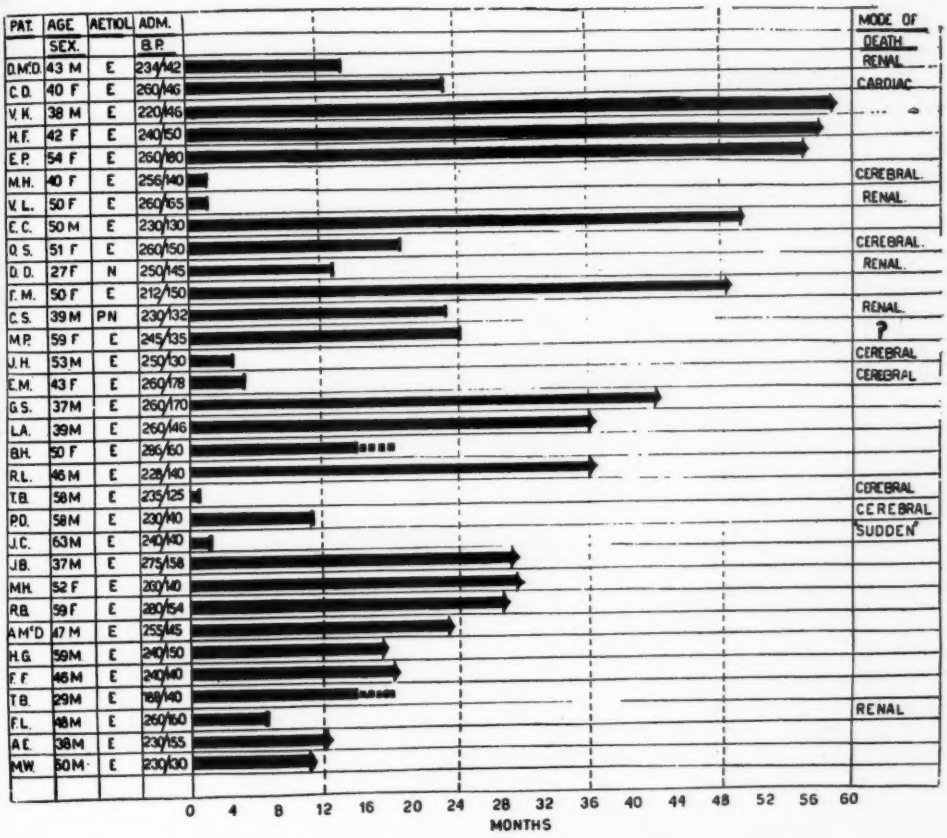


FIGURE VI

Survival in patients with hypertension with papillœdema (" malignant hypertension ") treated with ganglion-blocking drugs. An arrow head (→) at the end of the line representing survival indicates that the patient is still alive ; a vertical line (—|) that he has died ; and an interrupted line (— — —) that he has been lost sight of. E. = essential ; N. = nephritic ; P.N. = pyelonephritic

evidence that it is producing deleterious effects on the individual. In "malignant" hypertension the effects are readily noted, and the prognosis without treatment is so bad that treatment by the most effective means is urgently required.

The "relative risk" (that is, chance of death of hypertensive compared with non-hypertensive persons) is greater in young patients and in males (Frant and Groen, 1950; Palmer and Muench, 1953). Therefore, in young people, particularly men, with severe hypertension the use of ganglion-blocking drugs is indicated to attempt to forestall complications and possible progress to the "malignant" phase.

Sometimes there are hypertensive symptoms demanding rapid relief. The most pressing of these is paroxysmal dyspnœa due to left ventricular failure, which readily responds to reduction of blood pressure with ganglion-blocking agents. Frequent severe headaches may also demand treatment.

In most patients with benign hypertension symptoms are not severe, and the prognosis without treatment is so good that there is no urgent need for treatment. In general there would appear no need for treatment unless there is evidence from the clinical findings or special tests that the "hypertension" is producing organ damage or "hypertensive disease". This requires a careful appraisal of the condition of the cerebral, ocular, cardiac and renal fields. If these show no evidence of impaired function, treatment is not required, although it may be prudent, if the blood pressure is well above the average normal for the patient's age, to review his condition at regular intervals of about twelve months. Should there be evidence of hypertensive disease (as contrasted with manometric hypertension), some treatment is required. This does not necessarily mean the use of ganglion-blocking agents. The benefit from reduced blood pressure has to be weighed against the inconvenience and risks of the treatment. In most cases it is justifiable (and wiser) to commence with less drastic measures (such as the use of reserpine alone), and use ganglion-blocking agents only if the response is unsatisfactory.

The ganglion-blocking agents are contra-indicated in gross renal failure and should be used with care in the treatment of patients with any renal disease. They should also be used cautiously in the treatment of elderly patients and of those with evidence of cerebral or cardiac atheroma, in whom symptoms may occur from a sudden reduction in blood pressure. They should also be used with caution in the treatment of patients who have had abdominal operations.

*Which is the Best Procedure?*

Our experience has shown the superiority of methonium given by injection over methonium given orally. Injection of pentolinium (in the appropriate dose) is about as effective as injection of methonium and produces a similar proportion of severe side effects. However, pentolinium given orally (unlike methonium given orally) has proved effective in many patients. Better blood pressure control (without severe side effects) is obtained by the combined use of pentolinium and reserpine.

In severe hypertension, particularly in the "malignant" phase, the best method is probably to start with injections of pentolinium with the addition of reserpine if blood pressure control is not adequate or is achieved only at the expense of severe side effects. A change to pentolinium orally administered *plus* reserpine may be made as the hypertension is brought under control. (The apparent superiority shown in Table VI of pentolinium given by mouth *plus* reserpine in respect to blood pressure control is somewhat unfair, as most of the patients given this treatment had previously been well controlled with injections of pentolinium with or without reserpine.)

## SUMMARY

Ganglion-blocking agents (methonium compounds, pentolinium) have been used alone or in combination with reserpine in the treatment of 80 patients with severe hypertension over a period of five years.

The results of this treatment in 67 patients who have either died (21) or been followed for more than six months (46) are presented in detail.

Symptomatic relief occurred in patients with headache (42 out of 48), impaired vision (18 out of 40), exertional dyspnœa (21 out of 27), paroxysmal dyspnœa (14 out of 14), *angina pectoris* (5 out of 6), minor cerebral disturbances (9 out of 11), loss of energy (10 out of 21) and nervous tension (5 out of 13).

In the 21 fatal cases, death usually occurred within twelve months and was due to a cerebral vascular accident.

Among the 46 survivors, the overall degree of clinical impairment of eyes, brain or heart decreased, that of the kidneys remained steady.

The combination of pentolinium given orally and reserpine has been particularly favourable, usually producing good blood pressure control and symptomatic relief with few side effects.

The results of treatment with the ganglion-blocking agents has been particularly satisfactory in "malignant" hypertension. Twenty-three of 32 patients have survived for more than one year and 12 for more than two years.

The use of ganglion-blocking agents is indicated in the more severe forms of hypertension (particularly in the "malignant" phase), but, with the possible exception of severe hypertension in young people, it is not indicated for hypertension without evidence of hypertensive disease.

## ACKNOWLEDGEMENTS

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# THE TREATMENT OF RHEUMATOID ARTHRITIS WITH CYSTEAMINE (β-MERCAPTOETHYLAMINE)[1]

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CYSTEAMINE[4] (β - mercaptoethylamine; "Becaptan" (Labaz)) protects mice against otherwise lethal doses of X-radiation, and a single intravenous injection of 200 milligrammes of this drug often eliminates the symptoms of radiation sickness in human subjects undergoing deep X-ray treatment of malignant tumours (Bacq *et alii*, 1953). Cystamine[5] (Di (2-aminoethyl) disulphide) has similar properties and may be administered orally (Bacq, 1953).

During the course of studies of the mode of action of these drugs, which are of interest as possible protectants against nuclear radiation, it was found that they possessed an anti-inflammatory action, and in this respect they resembled certain adrenal steroids. Lecomte *et alii* (1953) showed that cysteamine reduced the response to intradermally injected tuberculin in Mantoux-positive human subjects, and also reduced non-specific chemical inflammatory processes in rabbits' skin. Long (1954) found that a single intraperitoneal injection of cysteamine given six hours before an intradermal injection of tuberculin in guinea-pigs sensitized to B.C.G. vaccine significantly reduced the size of the subsequent inflammatory reaction. Coulon *et alii* (1953) demonstrated that in rats the intraperitoneal injection of cysteamine in a dose of 50 milligrammes per kilogram reduced the local inflammatory reaction which followed the intraarticular injection of sterile kaolin.

On the other hand, there is evidence that cysteamine has no action resembling that of ACTH. After the intraperitoneal injection of cysteamine in rats, there is a fall in the ascorbic acid content of the adrenals, but there is no fall in the number of circulating eosinophile cells and no increased urinary excretion of corticosteroids (Van Cauwenberge *et alii*, 1953).

Despite these findings it was considered that the apparent anti-inflammatory action of cysteamine warranted a small-scale trial of this drug in the treatment of rheumatoid arthritis.

## MATERIALS AND METHODS

Cysteamine is unstable, and was supplied as the base in ampoules each containing 200 milligrammes dissolved in one millilitre of water, neutralized with hydrochloric acid to pH 6·5 and sealed under nitrogen. The drug was administered intravenously immediately after its removal from the ampoule. The dose used was 200 milligrammes twice a day for one, two or three weeks. Side effects occasionally noted during administration were pain at the injection site, a fall in blood pressure, transient loss of accommodation, and a metallic taste in the mouth.

### *Number of Patients Treated*

Four patients suffering from active rheumatoid arthritis were treated. One had previously been treated with cortisone and another with ACTH (Lovell *et alii*, 1953), and in these subjects it was therefore possible to compare the effect of cysteamine with that of cortisone and ACTH.

All four patients were kept in bed for at least one week before treatment was started. During this period measurements were made as described and continued during the treatment.

### *Assessment of Effects of Cysteamine*

Temperature and pulse rate were measured daily, and blood sedimentation rates were determined weekly. The following measurements were also taken daily: (i) the diameters of the proximal interphalangeal, wrist and knee joints; (ii) the range of movement of the wrist, elbow, shoulder and knee joints; (iii) joint

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[4] Cysteamine $HS–CH_2–CH_2–NH_2$ (β-mercaptoethylamine)

[5] Cystamine $S–CH_2–CH_2–NH_2$ / $S–CH_2–CH_2–NH_2$ (Di(2-aminoethyl) disulphide).

tenderness determined by a dolorimeter, measurement being made of the force required to produce pain when the instrument was applied to the skin over the proximal interphalangeal joints of the ten fingers; (iv) power, as estimated by grip, measured in millimetres of mercury.

## RESULTS

CASE I.—The patient was a male, aged fifty years, who had suffered for six years from arthritis, involving the ankles, knees, shoulders, elbows, wrists and fingers. He had been treated with cortisone for a short period two years previously. This had produced temporary relief of pain and tenderness, which had been followed by relapse after withdrawal of the drug. Since then he had been maintained on aspirin, codeine and phenylbutazone with alternating exacerbations and remissions.

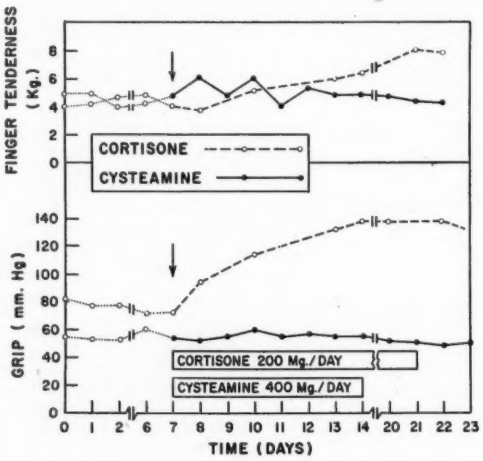


FIGURE I

The effect of cysteamine and cortisone on (i) the grip (mean of right and left hands), and (ii) joint tenderness (mean of the proximal interphalangeal joints of all ten fingers). Start of treatment indicated by arrows

He received one week's treatment with cysteamine, but then declined any more, as it had not produced any objective or subjective improvement, as compared with the earlier treatment with cortisone (Figure I).

CASE II.—The patient was a male, aged fifty-two years, who had suffered for fifteen years from arthritis involving the knees, ankles, wrists and fingers. He was given cysteamine for one week with no definite improvement, in contradistinction to the pronounced effect which had been noted when he was treated with ACTH three years previously.

CASE III.—The patient was a male, aged fifty-four years, suffering from polyarthritis of six months' duration involving the knees and the fingers. No improvement was found after two weeks' treatment with cysteamine.

CASE IV.—The patient was a male, aged thirty years, suffering from polyarthritis of twelve years' duration. There was slight subjective improvement during the initial period of rest, and during a week's treatment with a placebo of saline given intravenously, but there was no improvement during three weeks' treatment with cysteamine.

## DISCUSSION

In the dosage used, cysteamine had no discernible effect on the clinical features of rheumatoid arthritis in the four patients treated. However, the dose used (400 milligrammes per day—six milligrammes per kilogram per day) was relatively much less than that used by Coulon *et alii* (1953) when they demonstrated the anti-inflammatory action of cysteamine in experimental arthritis in rats—namely, 50 milligrammes per kilogram. It was also much less than that used by Long (100 milligrammes per kilogram) when he found that cysteamine reduced the response to tuberculin in sensitized guinea-pigs. However, the dosage used was the same as that which removes the symptoms of sickness in humans who are receiving deep X-ray therapy. But it is of interest that in animal experiments the anti-inflammatory dose of cysteamine is several times that required to give protection against X-radiation. Similarly the dose of 400 milligrammes per day, which removes the symptoms of human radiation sickness, may be much less than the dose necessary to demonstrate any anti-inflammatory action if indeed it is demonstrable. Increased dosage of cysteamine above 400 to 600 milligrammes per day is limited by side-effects and the difficulties of intravenous injection, but large oral doses of cystamine may be worth a trial.

## SUMMARY

Four patients with rheumatoid arthritis have been treated with cysteamine (β-mercaptoethylamine) given intravenously with a dose of 400 milligrammes per day. No effect on the symptoms or signs of the disease was observed.

## ACKNOWLEDGEMENTS

The writer wishes to thank Professor R. R. H. Lovell for his advice during this work and the preparation of this paper, and for permission to quote his work as a basis for the comparison of cortisone and cysteamine. He is grateful to Professor G. W. Pickering for his criticism, and to Dr. R. H. W. Britton, of Horlicks, Limited, for making available the "Becaptan" used in these experiments.

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# THE DEVELOPMENT OF DIFFUSE NUTRITIONAL FIBROSIS IN THE LIVER OF RATS[1]

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ALTHOUGH the use of the word cirrhosis is often critized today, it is here regarded as meaning a condition of the liver in which there is evidence of death and regeneration of liver cells, distortion of lobular architecture and fibrosis, but no ætiological factor is denoted. The condition has many causes and many paths of development. Nutritional deficiency is one cause recognized clinically, and much experimental work substantiates this; but human and animal observations cannot be correlated closely. Not only are many of the anatomical features poorly understood, but also the mechanisms of cell damage from nutritional deficiency are not yet elucidated. In the earlier stages of the development of cirrhosis, before the liver architecture becomes significantly distorted, the term fibrosis can be more accurately applied, and Himsworth (1950) prefers to use this term as a general alternative to cirrhosis; this overcomes many difficulties. In the present paper an attempt has been made to elucidate some of the features of diffuse nutritional fibrosis in the rat liver.

## MATERIALS AND METHODS

Rats of mixed indeterminate strain, bred locally, were fed the following diets:

*Diet I*

| | |
|---|---|
| Casein | 7·0% |
| Gelatin | 11·0% |
| Celluflour[1] | 2·0% |
| Maize starch | 10·0% |
| Dextrin | 10·0% |
| Sucrose | 43·37% |
| Vitamin powder | 1·0% |
| Salt mixture | 3·0% |
| a-Tocopherol | 0·01% |
| Vitamins *A* and *D* concentrate | 0·02% |
| Beef fat | 10·0% |
| Maize oil | 2·0% |
| Cystine | 0·3% |
| Glycocyamine | 0·3% |

*Diet II*

| | |
|---|---|
| Casein | 5·0% |
| Celluflour[1] | 2·0% |
| Sucrose | 83·66% |
| Vitamin powder | 1·0% |
| Salt mixture | 3·0% |
| a-Tocopherol | 0·01% |
| Vitamins *A* and *D* concentrate | 0·02% |
| Maize oil | 5·0% |
| Cystine | 0·3% |

The vitamin powder used was that described by Ridout, Lucas, Patterson and Best (1954), with the addition of 300 microgrammes of vitamin $B_{12}$ per 100 grammes; the salt mixture was that described by Beveridge and Lucas (1945). The vitamins *A* and *D* concentrate contained 200,000 international units of vitamin *A* and 50,000 international units of vitamin *D* per gramme.

The methionine content of Diet I was estimated as 0·29% and of Diet II as 0·15%. Diet I was moderately deficient in nearly all essential amino-acids, and Diet II was severely protein deficient. Casein was prepared as follows: commercial casein was soaked for twenty-four hours in 50% ethyl alcohol, squeezed "damp-dry" in a pharmaceutical press, dried thoroughly in an oven at about 90° C. and then reground to a fine powder.

### *Technique*

The livers of 35 rats, which had been fed on one or both of these diets for different periods, were studied. Each was anæsthetized with ether, its abdomen and thorax were opened, and into the still pulsating left ventricle were injected 500 units of heparin in one millilitre of water. The rat was then exsanguinated by opening the superior or the inferior *vena cava*. In most cases the liver was injected via either the portal vein or the hepatic vein,

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[1] "Alphacel", obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, United States of America.

an opaque green ink being used[1]. This ink was found very satisfactory as an injection mass, as its suspended particles did not dissolve during subsequent tissue processing, and the colour resisted all fixing, bleaching and staining procedures. It was preferred to carbon particle suspensions, as the green contrasted well with the black of silver-impregnated reticulin. In a dilution of two parts of ink to one part of water, the ink usually filled as far as the smallest venous branches or radicles, but only occasionally extended into sinusoids. The method was valuable (i) in distinguishing vessels of the hepatic from those of the portal systems in silver-impregnated sections, where nuclear counterstaining is difficult, and (ii) in helping to distinguish large fat vacuoles and fatty cysts from small vessels in cross section.

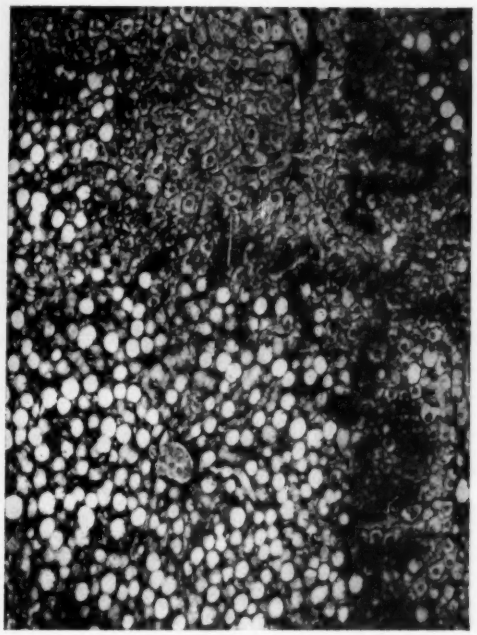

FIGURE I

Photomicrograph of a section of a rat liver showing centrolobular fat in choline deficiency. The periportal cells are swollen and pale. There is no condensation of reticulum. (Lendrum's reticulin stain, ×60)

The hepatic venous system was filled by ligating the inferior *vena cava* below the liver and above the right renal vein and cannulating it in the thorax. The portal vein was cannulated directly. The ink was injected manually with a hypodermic syringe, care being taken to exclude all air. Gentle pressure and slow introduction of the ink enabled the venous systems to be fairly completely filled without intrahepatic rupture of a vessel. The livers were removed and blocks cut; these were fixed in formalin and embedded in paraffin. Sections from all were stained with hæmatoxylin and eosin, van Gieson's stain for collagen, and silver impregnation as described by Lendrum (1951) with carmalum counterstaining was used for reticulin.

[1] Dark Green "Velour", Show Card Colour; Caldwell's Proprietary Limited, South Melbourne.

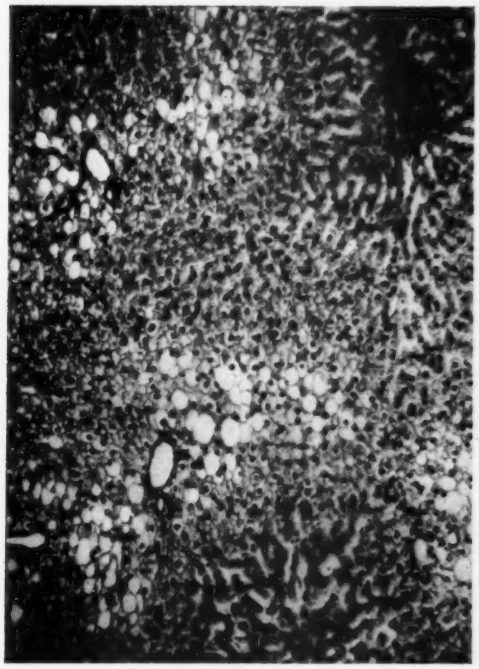

FIGURE II

Photomicrograph of rat liver showing periportal fat assumed to be due to protein deficiency. Central veins have been filled with green ink. Transition from the "clear-cell" appearance to manifest fatty change is shown. Rat fed on Diet II for seventy-three days. (Hæmatoxylin and eosin, ×60)

Two types of liver cell damage, occurring singly or in combination, were observed. They could be recognized readily by the maximal distribution, centrolobular or peripheral, of fat accumulation. The first type was the primarily centrologular fatty change characteristic of choline deficiency (Figure I) with, in those rats which had been given the diet for prolonged periods, the presence of fatty cysts as described

by Hartroft (1950, 1950*a* and 1951). The second type was periportal in situation. In five organs there was considerable periportal fat accumulation and no centrolobular fat (Figure II); but in nearly all livers, for a variable distance away from the portal triads, the periportal cells were swollen and pale but

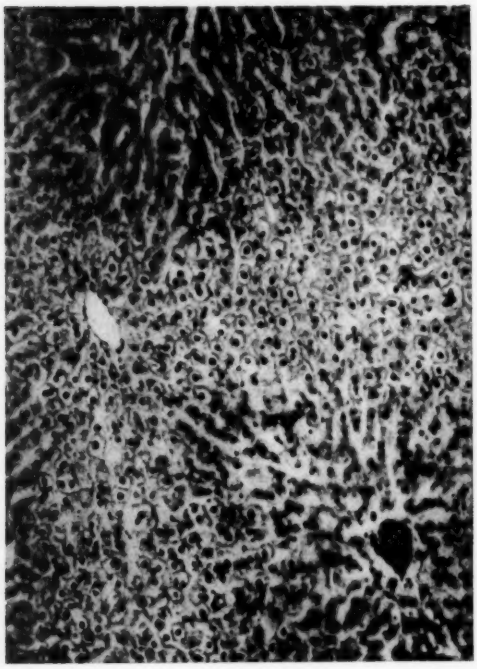

FIGURE III

Photomicrograph of rat liver showing the striking "clear-cell" appearance in the periportal zones—assumed to be due to protein deficiency. Central veins filled with green ink. (Hæmatoxylin and eosin, ×75)

with a distinct outline; here the lumina of sinusoids could rarely be seen between them (Figure III). In some cells, multiple small intracytoplasmic vacuoles could be seen. A gradual transition from the swollen pale cell to that containing one large fat vacuole was demonstrable. Fatty cysts were not seen in the periportal zones.

The vascular and connective tissue changes associated with these two types of damage, for convenience of presentation, are considered separately.

*First Type*

The gradual development of these changes associated with the first type of liver cell damage could be followed in detail and for convenience in description, division is made into four main stages.

The first stage was simply a dilatation of sinusoids as they enter the central veins, associated with atrophy or disappearance of adjacent parenchymal cells. Sinusoids round a central vein in a normal rat liver were clearly outlined by their delicate reticulum (Figure IV). A similar appearance was found in the liver of a rat fed on Diet II for two to three weeks, but here the pattern was modified by the considerable centrolobular accumulation of fat (Figure V). After another five weeks this was modified further (Figure VI); in this liver (Figure VI) there was an extensive periportal "clear-cell" appearance and a considerable amount of periportal fat, but fat was absent

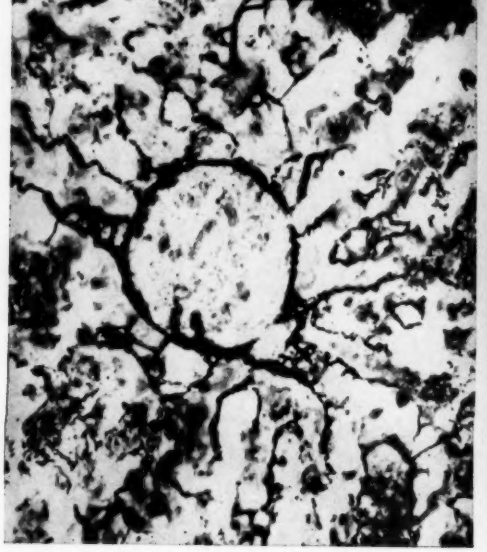

FIGURE IV

Photomicrograph of rat liver showing the distribution of reticulum around a central vein of a normal rat liver. (Lendrum's reticulin stain, ×500)

from the centrolobular zones. Although sinusoids around the central vein were sometimes greatly dilated, usually there was no increased density of reticulum.

The second stage was the appearance of an increased density of reticulum around the central veins. Unusually thick bands occurred

in the reticulum of dilated sinusoids, and dense masses of reticulum appeared between cells. In animals fed on Diet I for about twenty weeks, these changes were found, and also there was a large amount of centrolobular fat with a number of fatty cysts (Figure VII). At this stage sections counterstained with van Gieson's stain showed for the first time the presence of thin collagen bands, sometimes related to sinusoids and sometimes not, running out from the central veins; sometimes thin basket-like arrangements of collagen fibres were seen surrounding sinusoids.

The third stage was characterized by the extension of condensed reticulum well out from the central veins. The condensation conformed to the sites of maximum fatty change, and it loosely connected neighbouring central veins

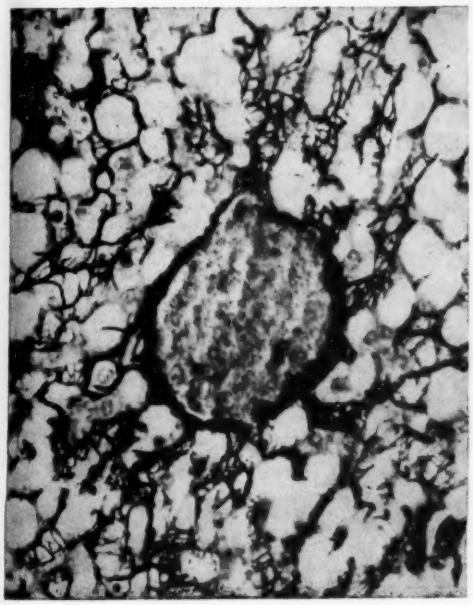

FIGURE V

Photomicrograph of rat liver showing a central vein and its surrounding reticulum, from a liver showing early centrolobular fatty change. The reticulum density is within normal limits. Rat fed on Diet II for seventeen days. (Lendrum's reticulin stain, × 500)

(Figure VIII). The centrolobular areas showed considerable fatty change and loose networks of condensing reticulum extending between central veins. Although in some cases a well-defined reticulum condensation was seen in the periportal area (Figure IX), the portal triads were clearly distinct from this reticulum condensation and the reticulin density around them was usually normal. This liver (Figure IX) contained minimal centrolobular fat, but there was an extensive periportal " clear-cell " appearance. van Gieson's stain at this stage demonstrated a greater amount of collagen around central veins, and occasional thin incomplete

FIGURE VI

Photomicrograph of rat liver showing dilatation of sinusoids as they enter a central vein. The central vein and many of the sinusoids have filled with green ink. Rat fed on Diet II for forty-five days. (Lendrum's reticulin stain, × 500)

bands running between neighbouring central veins.

A further feature, making its appearance at about the third stage, was the development of what may be called " central vein systems ". The central vein became represented by a group of veins of different diameters, and the original vessel was often no longer distinguishable (Figure X). These new veins were grouped along the lines of reticulum condensation for variable distances; obviously some of the dilated sinusoids of the first and second stages had become obliterated, whilst in others, which had enlarged, reticulin had accumulated in the walls and they approximated to the size of the original central vein.

The fourth stage was the obvious progression from the third stage; here the reticulum had condensed into thick septa running between central veins, sharply outlining the triangular "portal" lobules, but not connecting with portal triads (Figure XI). This occurred in animals fed on Diet I for about twenty-seven

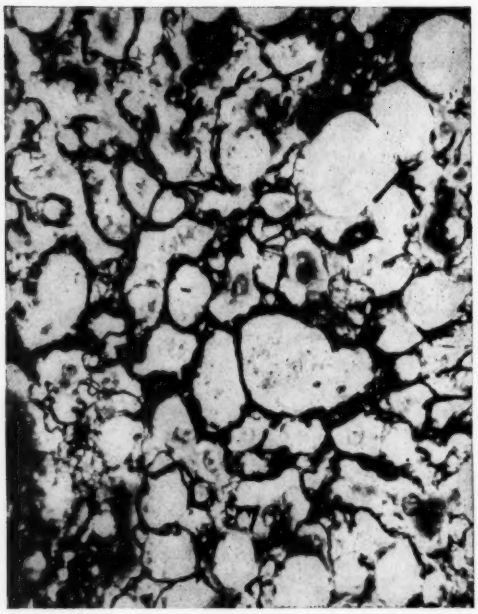

FIGURE VII

Photomicrograph of rat liver showing dilatation of sinusoids and early reticulum condensation in a liver showing fairly severe centrolobular fatty change. Rat fed on Diet I for 139 days. (Lendrum's reticulin stain, ×500)

weeks; the livers were grossly fatty. Van Gieson's stain showed that these septa were intimately associated with or were being transformed into collagen. Modification of the central vein systems was seen at this stage, with apparent disappearance of some vessels and enlargement of others, and not only were there usually several vessels at the sites of the original central veins, but often many of the newly formed vessels were seen scattered along the septa (Figure XII).

*Second Type*

The vascular and connective tissue changes of this second type of nutritional liver damage were associated with the accumulation of fat in the periportal zones. A considerable amount of periportal fat was seen, but centrolobular fatty change was not found in animals fed on Diet II for ten weeks (Figure II). There was early condensation of reticulum around the portal triads (Figures XIII and XIV). This condensation was not only seen in association with well-developed periportal fatty change, but also occurred to some degree in association with an extensive periportal "clear-cell" appearance (Figure IX). These septa of condensed reticulum appeared to extend out gradually through the "portal" lobule and to subdivide it, and once again van Gieson's stain showed collagen distributed in the same positions as the condensed reticulum. However, the septa did not run through the lobules

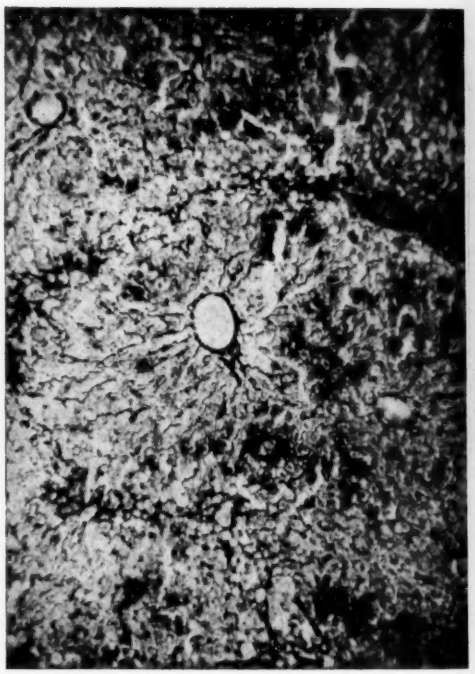

FIGURE VIII

Photomicrograph of rat liver showing early condensation of reticulum at the periphery of a portal lobule. The central veins have filled with green ink. The portal triad is seen unassociated with the reticulin condensation. (Lendrum's reticulin stain, ×50)

to connect with central veins, but followed the lines of maximal cell damage; they ran towards neighbouring portal triads, reaching the periphery of the "portal" lobule at a point intermediate between central veins. In some, considerable amounts of condensed reticulum and collagen extended out from both portal

triads and central veins, and the relation between the septa of portal origin and the central veins could usually be seen (Figure XV). The degree of damage to the liver was not always proportional to the length of time during which the animal had been on the diet, as was shown by some which were fed on the diets for sixty weeks and yet showed minimal fibrosis. The late stage of diffuse fibrosis, observed in rats fed on Diet I for seventy weeks (Figure XVI), fulfilled the criteria for cirrhosis. Connective tissue septa ran irregularly through the parenchyma apparently connecting both portal and central vessels; sometimes there was a vessel at the point of intersection of septa and sometimes not.

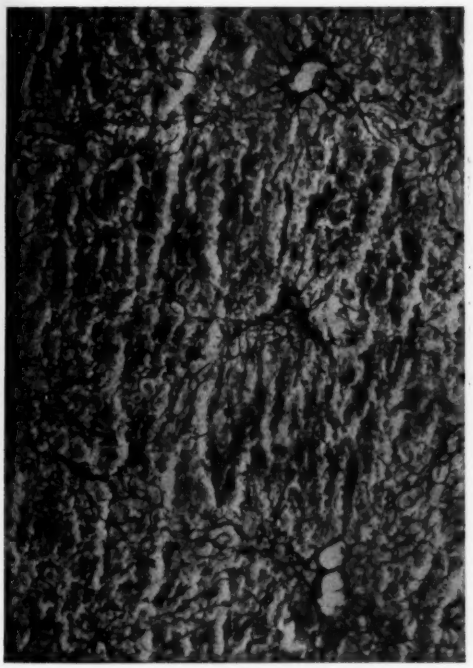

FIGURE IX

Photomicrograph of rat liver showing early condensation of reticulum at the periphery of a portal lobule. There is also some condensation around the portal triad. This liver showed a severe periportal "clear-cell" appearance, but no periportal fat. (Lendrum's reticulin stain, ×50)

## DISCUSSION

At least three nutritional causes for liver injury are recognized in rats—the animals most extensively studied experimentally in this respect. First, dietary deficiency of thio-amino-acids and α-tocopherol will produce massive necrosis (Glynn, Himsworth and Neuberger, 1945; György, 1947; Himsworth and Lindan, 1949); secondly, protein deficiency in the presence of adequate lipotropic factors, thio-amino-acids and α-tocopherol will cause periportal fatty change (Best, Hartroft, Lucas

FIGURE X

Photomicrograph of rat liver showing "central vein system" from the same liver as Figure IX. Subsidiary veins are seen scattered along the lines of reticulum condensation. Same rat as in Figure IX. (Lendrum's reticulin stain, ×50)

and Ridout, 1955); and thirdly, deficiency of choline and other lipotropic factors will produce severe fatty change which is primarily centrolobular in site (Glynn and Himsworth, 1944). The development of cirrhosis following the first and third causes of liver-cell injury is well recognized, the first causing nodular hyperplasia and the third causing diffuse fibrosis.

The way in which fatty change is related to cell death is uncertain, and, indeed, that the relation is a direct one in the development of diffuse nutritional fibrosis in man has been strongly challenged (Dible, 1951). Popper, Szanto and Elias (1955) have shown that the fibrosis begins round the portal triads in human

fatty livers, in contrast to its centrolobular beginning in choline deficient rats ; they suggest that the human fatty liver is more sensitive than the normal liver to infections and other injuries. In rats, however, a more constant relation has been shown, and Himsworth (1950) claims that while livers containing

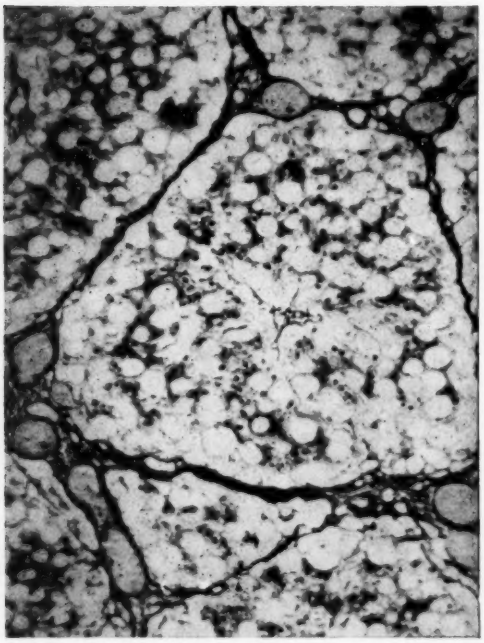

FIGURE XI

Photomicrograph of rat liver showing fairly late stage in the fibrosis surrounding portal lobules. The portal triad is unassociated with the reticulum condensation. Numerous central veins filled with green ink are seen at the periphery of portal lobules. Rat fed on Diet I for 188 days. (Lendrum's reticulin stain, ×50)

30% fat will develop fibrosis in one hundred days, those containing 15% fat develop it only after three hundred days. These high levels of liver fat content are rarely seen in man. It may be that some degree of cellular fatty change must be reached before death occurs, or that fat accumulation is simply an index either of other metabolic disturbance or of increased susceptibility to other damaging agents. Hartroft (1950) has described atrophy and fibrous replacements of the cells surrounding fatty cysts, and Glynn, Himsworth and Lindan (1948) believe that death of cells results from ischæmia when swollen, fat-laden cells occlude sinusoids proximally.

For the present study rats were fed, for various lengths of time, on choline-deficient diets based on those used by Best *et alii* (1949 and 1955). These diets were also deficient in protein. Choline can be eradicated from a diet quite easily, but it is far more difficult to lower selectively the methionine content. If the figures given by Farris and Griffith (1949) are taken for the essential amino acid requirements of rats, and if casein is used as the source of protein, a minimum of 18% must be included in the diet to avoid protein deficiency. Even a completely choline-free diet containing this quantity of casein results in but little increase in liver fat (Best *et alii*, 1955). A similar result is obtained with most other proteins, an exception being raw soybean meal (Greenberg,

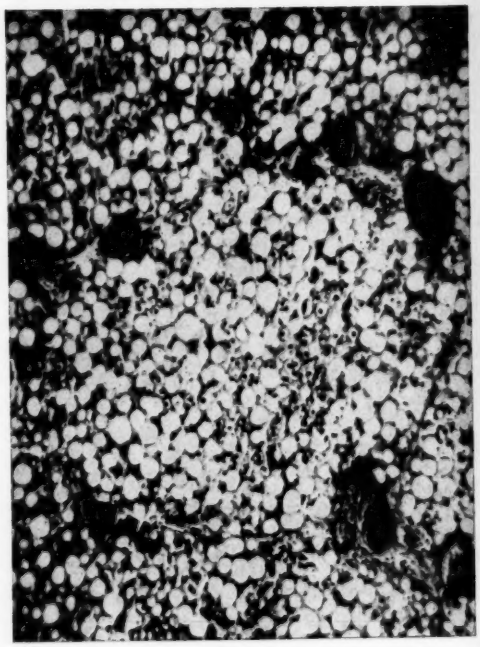

FIGURE XII

Photomicrograph of rat liver showing a portal lobule from the same liver as in Figure XI, with collagen fibres running between numerous central veins at its periphery. (Van Gieson's stain, ×50)

1951). Although this contains adequate methionine, unless the meal is previously heated, the methionine is largely unavailable to the animal. An expensive form of selectively lowering the methionine content is the use of individual amino acids ; but a probably acceptable method for overcoming the problem

is to use a methyl group competitor such as glycocyamine.

Considerable difficulty was experienced in producing fatty livers consistently with these diets. Although choline was virtually absent according to determinations by means of the Reineckate method described by Glick (1944), methionine was suitably deficient as estimated from protein amino-acid composition tables (Greenberg, 1951), and other possible sources of lipotropic substances such as betaine were eliminated, the fat content of the livers of rats killed from time to time varied considerably. Coprophagy was considered as a source of lipotropic factors; but its prevention by placing rats in confined, annular grid-bottomed

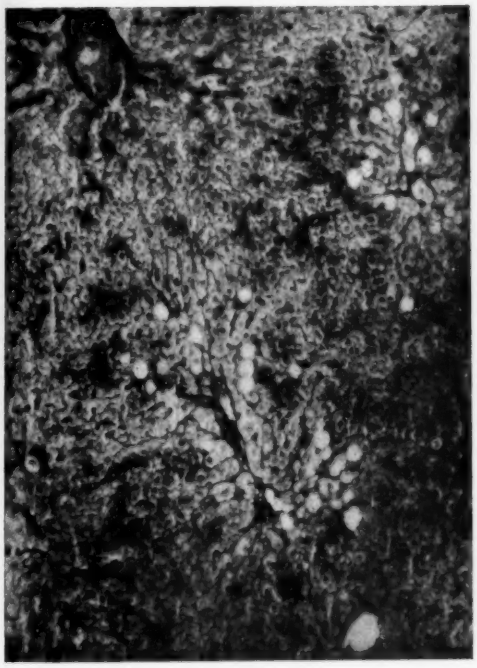

FIGURE XIII

Photomicrograph of rat liver showing reticulum condensation around portal triads in association with periportal fatty change. The centrolobular zones appear normal. (Lendrum's reticulin stain, ×50)

cages made no difference; incidentally this, as a complication, has not been mentioned by other writers. One reason for the discrepancies found was possibly that initially the casein, after being washed and dried, was not ground sufficiently finely, and some rats learned to select it from the diet. However, grinding all components of the diet to a very fine powder did not overcome the problem completely; as the livers of some rats showed what was taken to be evidence of protein deficiency without significant increase in centrolobular fat, it was assumed that in these centrolobular fat had disappeared at a very much earlier stage

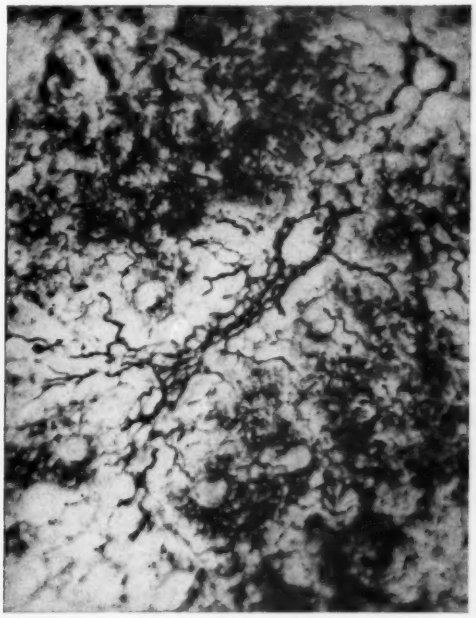

FIGURE XIV

Photomicrograph showing a portal triad from the same liver as Figure XIII. (Lendrum's reticulin stain, ×350)

than usual. It was decided that the main reason for these results was that the rats, of mixed, indeterminate strain, managed to compensate to a varying extent for choline deficiency. Although the degree of change seen could not be related closely to the protein and/or choline content of the diet, or to the length of time a rat was kept on the diet, a wide variety of stages of nutritional damage to liver was available for study.

The first type of damage—namely, the centrolobular accumulation of fat — was characteristic of choline deficiency (Figure I), and was observed at many stages of development. The second type (Figures II and III), from consideration of the observations of Best *et alii* (1955), was considered to be a manifestation of protein deficiency. These workers mention only the appearance of periportal fat

in this connexion, but the " clear-cell " appearance could be considered a preliminary stage to the appearance of large fat vacuoles in this zone. Ashburn, Endicott, Daft and Lillie (1947) point out that the liver can be regarded as being composed of either portal or hepatic lobules—that is, of lobules centred on the

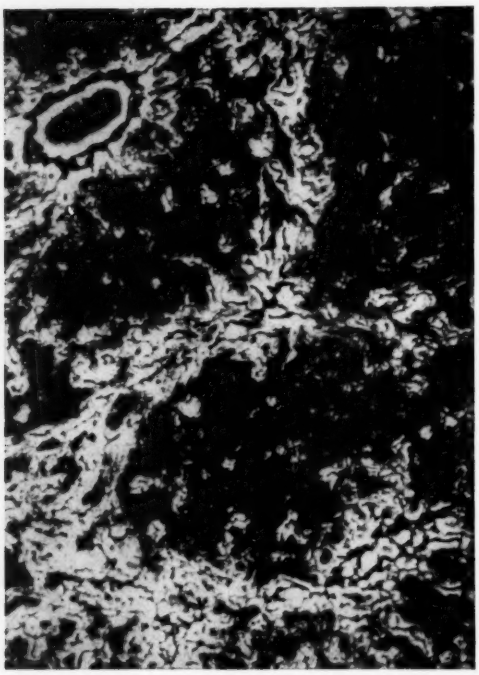

FIGURE XV

Photomicrograph showing central veins (situated in the top right and bottom left corners) and a portal triad (in the middle). There is reticulum condensation in association with both, but the lines radiating from the portal triad are seen to run between central veins, not towards them. Rat fed on Diet I for 421 days. (Lendrum's reticulin stain, ×50)

portal triads or on the central veins. However, in man, this concept is artificial, and connective tissue septa can develop at the periphery of either one or the other lobule depending on the precise site of the injury. In choline deficiency in rats the injury is at the periphery of the triangular portal lobule, and initially, at any rate, the fibrosis takes the form of septa surrounding these lobules, unconnected with the portal triads. As the connective tissue changes associated with the periportal damage were recognized only in retrospect, and the more severely protein-deficient Diet II was not introduced until later in the study, they could not be followed as well, or in as many livers, as the centrolobular damage. However, it is suggested that in rats protected from massive necrosis of the liver by inclusion of α-tocopherol and cystine in the diet, protein deficiency will cause tissue damage at the periphery of the hexagonal hepatic lobules resulting in fibrosis in this situation ; if choline deficiency is avoided, it may be possible to produce a pure lesion demonstrating septa surrounding these lobules, not connecting with central veins and without associated centrolobular fibrosis. Whether rats will survive protein deficiency for sufficiently long for this to occur is unknown. In diffuse nutritional fibrosis in man the primary change is the development of septa of portal origin in

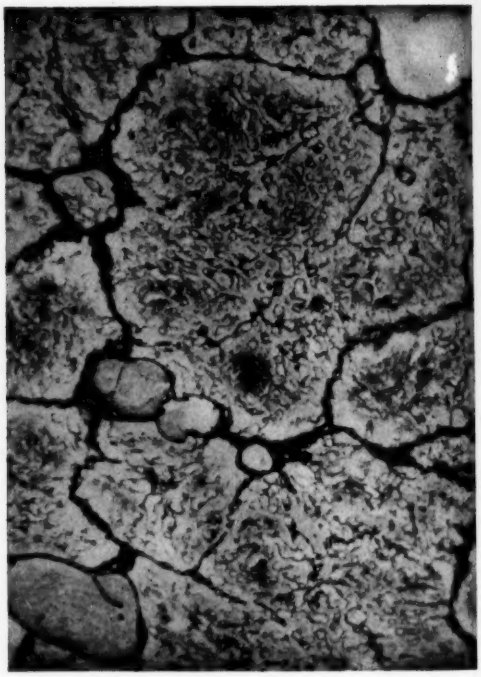

FIGURE XVI

Photomicrograph showing a late stage in the development of diffuse fibrosis, where the architecture is distorted. Central veins are filled with green ink. (Lendrum's reticulin stain, ×50)

association with periportal inflammation and areas of necrosis. The cause of periportal tissue damage has been thought to be probably " toxic " or infective, in a liver of impaired " resistance " to such damage by reason of its fatty change (Popper *et alii*, 1955). That protein deficiency, apart from causing massive

necrosis, can apparently produce periportal damage in rats with resulting septum formation suggests that this may be a factor in man.

In the later stages of nutritional fibrosis, septa appear to connect portal triads and central veins (Figure XVI); from the foregoing discussion this would not be expected to occur, since septa connecting portal triads should cross strands connecting central veins at a point between vessels. The explanation of this discrepancy can be found by considering the development of the "central vein systems" or subsidiary central veins observed and described earlier. These veins, scattered along the septa connecting central veins, may often lie in the paths of septa connecting portal triads, and at this late stage original central veins cannot be distinguished from subsidiary veins. To what extent vascular changes, similar to those described for the centrolobular region, may develop in the portal regions, was not studied. However, the presence of vessels in septa radiating from the portal triads in man, injectable via the portal vein, has been demonstrated (Popper, Elias and Petty, 1952). Similarly, communications between portal branches and central veins, although probably they are between portal veins and subsidiary central veins, have also been shown (Moschcowitz, 1948). Thus, in this regard, the observations made in rats in experimental conditions resemble those made in man.

In the experiments described here, inflammation, as evidenced by collections of inflammatory cells and by fibroblast proliferation, was not observed, and the fibrosis appeared in relation to what was almost certainly condensation of preexisting reticulum. As cells between neighbouring sinusoids disappeared, some sinusoids dilated and reticulin accumulated in their walls, whilst others collapsed. In agreement with Popper and Elias (1955), no convincing evidence for new formation of reticulin was seen other than that presumed to occur in association with regeneration of parenchyma. Kramer and Little (1953) state that reticulin, which is probably the same as the basement membrane of an epithelial surface, is made up of a feltwork of fine fibrils lying in an amorphous matrix, and it may have the form of membrane or of honeycomb or of an open network. Fibrils show a cross striation of the order of 650 Å which is typical of collagen; chemical and X-ray diffraction studies demonstrate their close similarity to collagen. It is considered by these workers and by Robb-Smith (1952) that reticulin can change to collagen, the most important change being the loss of the amorphous matrix. It is probable that the high carbohydrate content of the matrix is responsible for the argyrophilia of reticulin.

Popper and Elias (1955), however, claim that collagen membranes arise newly-formed within the framework of condensed reticulum; but the distribution of collagen so closely patterned the distribution of the thicker reticulin fibres that, from this study, maturation of reticulin into collagen seemed a far more acceptable concept of its origin.

## SUMMARY

The development of diffuse fibrosis in the livers of rats fed on diets deficient in choline and protein, but containing α-tocopherol and cystine (to give protection from massive necrosis), was studied.

Choline deficiency produces fibrous septa which outline the portal lobules by connecting neighbouring central veins; it has been observed that protein deficiency produces fibrous septa developing in relation to portal triads, and it is suggested that these can ultimately outline the hepatic lobules by connecting neighbouring portal triads.

The development of subsidiary central veins running in the septa surrounding portal lobules is described. The fibrous connexions which appear to connect portal triads and central veins, seen in the late stages of nutritional fibrosis, are considered to be the result of the intersection of the two types of septa at points intermediate between neighbouring original central veins, where subsidiary central veins are situated.

The fibrosis develops in relation to condensation of preexisting reticulum, and the relation between reticulin and collagen is discussed. It is considered that the collagen develops as a result of maturation of the condensed reticulum.

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